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VITAMIN B_1
(THIAMIN)
AND ITS USE IN MEDICINE

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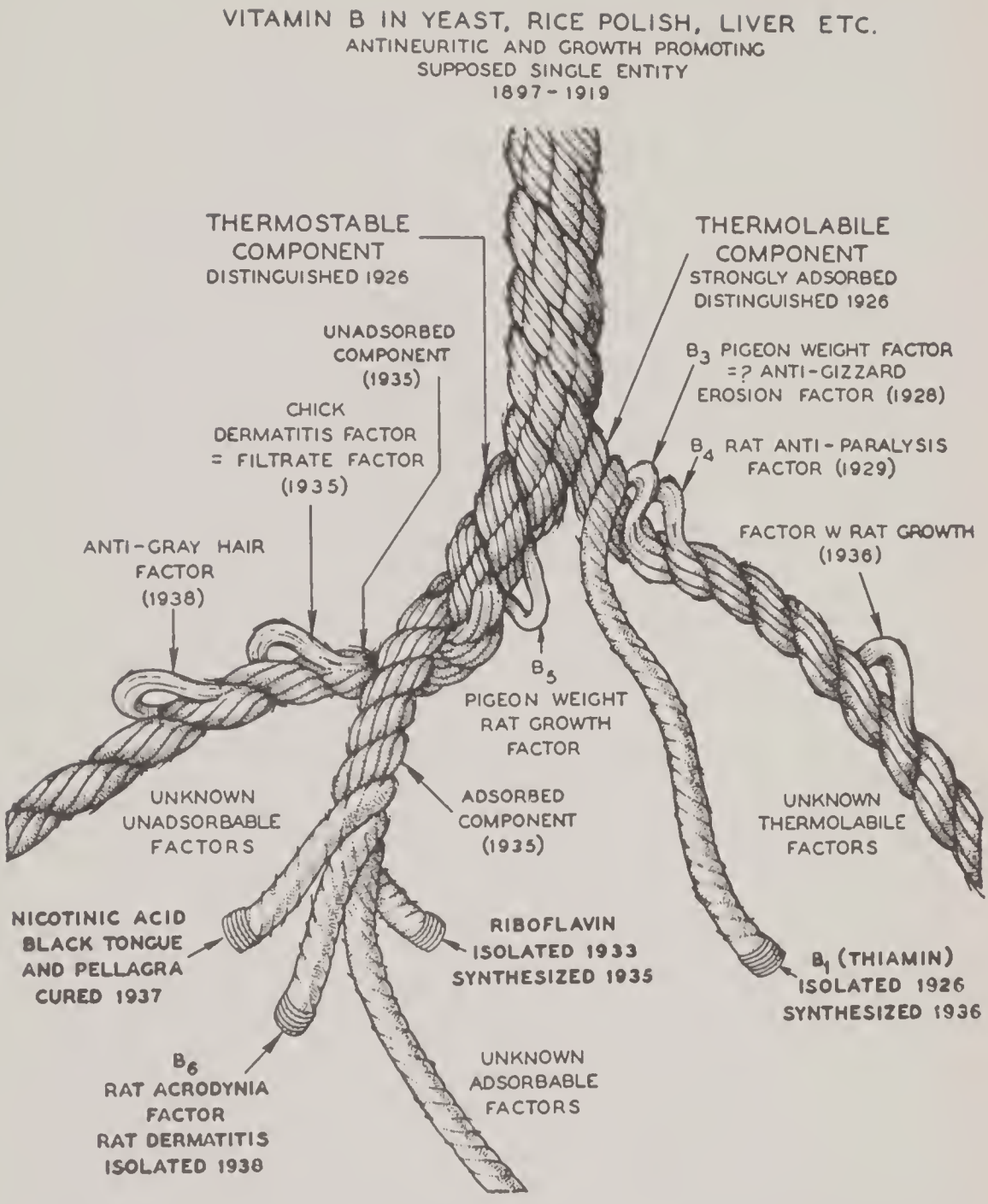

Differentiation of the Components of Vitamin B Complex. The components that have been isolated in a pure state are indicated in heavy lettering; those which have been recognized only by the discrepancies in physiological properties of various crude extracts are indicated as loops; the possible still unknown factors are divided into three main strands according to the principle of adsorbability which has been so useful in differentiation experiments. See page 133.

VITAMIN B_1
(Thiamin)

AND ITS USE IN MEDICINE

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470

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Vitamin B1 and

FOREWORD

In no other area of medical knowledge has there been recorded such a far-reaching advance as has been made during the past two decades in the field of nutrition. Pointing the way to the development of a healthier, more vigorous race, this advance marks an epoch in man's progress.

Among the discoveries involved those related to Vitamin B_1, of which Dr. Williams and Dr. Spies write, are perhaps of greatest interest, for it now appears that the chief function of this vitamin is to regulate the most fundamental of all life processes, cell respiration. This is of immediate interest to the clinician because of the recent demonstration that many forms of neuritis and allied disorders are directly related to impairment of this function. These discoveries hold, too, an additional interest for the thoughtful physician because of the orderly manner in which they have been developed. This knowledge has come, not as the result of a single brilliant discovery but through a long series of carefully recorded clinical observations followed by painstaking laboratory research.

First, centuries ago, the Chinese wrote of beriberi. In modern times when this disease had incapacitated a large part of the Japanese navy, the clinical observations of Takaki led to an early intimation of its nutritional origin, and a little later animal experiment in the Dutch East Indies made its first contribution by demonstrating a similar effect upon fowls of the prevailing type of unbalanced diet. Great progress was next made through significant observations covering a period of years reported from the Malay States and the Philippines by groups of English and American physicians. Following this came the studies of American laboratory workers who soon were able to demonstrate the general chemical nature of the missing, or "antineuritic," factor. Then followed a series of carefully planned biochemical researches culminating in the notable success of one of the authors of this book, first in determining the structure, then in synthesizing this vitamin. The further identification of this substance as the specific factor which is missing in beriberi has completed the story. It is an inspiring story—a story of medical progress in which, with due observance of traffic rules, the ultimate destination has been reached, not by short-cuts, but by long, laborious travel over a well-worn highway.

James S. McLester

PREFACE

THE chemical structure of the antineuritic vitamin is now known, and its artificial synthesis has recently been achieved. A milestone in its history having thus been passed, it seemed appropriate to review and summarize in convenient form the knowledge of this important substance. In doing so, the authors have thought it wise to divide the material into two parts. In Part I we have recorded in concise, accessible form the available information which we consider should be of immediate value in the practice of medicine. As our knowledge of the clinical aspects of the subject is new and includes much that has not yet been fully confirmed, a more elaborate treatment promised to be less useful than a brief one.

In Part II, which deals with history and with experimental evidence derived from laboratory work, a different course seemed advisable in order best to meet the needs of the majority of readers. In this field a vast, often confusing and sometimes conflicting, literature has accumulated which the busy person can scarcely hope to scan comprehensively. We have therefore undertaken a critical survey of it, selecting what seems best established, endeavoring to reconcile viewpoints which appear to be in conflict and attempting the development of a consistent philosophy of the whole subject. While many points of uncertainty remain, we have been somewhat surprised and greatly gratified at our success in resolving, at least to our own satisfaction, many of the moot questions which at the outset appeared somewhat appalling. We hope that the treatment of these disputed topics will appear reasonable to our readers and that the second part will prove serviceable, not only for the physician who wishes to scrutinize the original evidence, but also for the biochemist, biologist, and student of nutrition.

We gratefully acknowledge our indebtedness to many colleagues who have not only offered constructive criticism of several of the chapters but have in many instances provided us with unpublished data or with advance copies of manuscripts. Special thanks are due to Miss Elisabeth Zschiesche, Miss Margaret Higgins, Mr. Robert E. Waterman and Mrs. K. M. Jones for assistance in the composition, proofreading and indexing.

ROBERT R. WILLIAMS
TOM D. SPIES

CONTENTS

PART I

Vitamin B_1 (Thiamin) in the Practice of Medicine

CHAPTER PAGE

I. General Clinical Considerations 3

Frequency of Borderline Deficiencies, 3. Components of Vitamin B Complex, 3. Factors Operating to Produce a Deficiency, 4. Systems Involved and Lesions Observed in Deficiency Disease in Adults, 5. Vitamin B_1 Deficiency in Infants and Children, 5. Objective Changes Associated with Vitamin B_1 Deficiency, 6. Predisposing Causes of Vitamin B_1 Deficiency, 7. Prodromal Symptoms of Vitamin B_1 Deficiency, 8. Alimentary Tract in Vitamin B_1 Deficiency, 8. Cardiovascular System in Vitamin B_1 Deficiency, 8. Classification of Causes of Edema, 8. The Nervous System in Vitamin B_1 Deficiency, 9. Causes of Neuritis, 10.

II. Beriberi and Closely Related or Identical Diseases 16

Synonyms, 16. Definition, 16. Incidence, 17. History, 17. Etiology, 18. Signs and Symptoms of Clinical Beriberi, 19. Correlation of Symptoms with Degree of Deficiency, 20. Nervous System, 21. Cardiorespiratory Symptoms, 21. Edema and Other Symptoms, 21. Diagnosis, 22. Prognosis, 23. Ship Beriberi, 24. Epidemic Dropsy, 25. Hunger Edema, 26. Sporadic Occurrence of Beriberi, 26.

III. Vitamin B_1 and the Polyneuritis of Sundry Diseases 34

Polyneuritis in the Chronic Alcoholic Addict, 34. Relationship between Lack of Vitamin B and "Alcoholic" Polyneuritis, 35. Studies with Crystalline Vitamin B_1, 38. Diagnosis, 40. Polyneuritis of Pregnancy, 41. Malnutrition and the Polyneuritis of Pregnancy, 41. Studies with Crystalline Vitamin B_1, 43. Diagnosis, 44. Korsakoff's Syndrome, 44. Pellagra, 44. Sprue, 45. Pernicious Anemia, 46. Colitis, 47. Diabetes, 47. Tuberculosis, 49. Porphyrinuria, 49. Senility, Tumors and Cirrhosis of the Liver, 50.

CHAPTER PAGE

IV. Pathology and Pathological Physiology of Vitamin B_1 Deficiency in Animals and Human Beings 57

Pathology in Animals, 57. The Nervous System Lesions, 57. The Cardiovascular System, Edema and Serous Effusions, 59. The Gastro-intestinal Tract, 59. Internal Secretions, 60. Pathology in Human Beings, 60. Nervous System, 61. The Heart, 62. Edema, Serous Effusions, and Other Findings, 63. Pathological Physiology, 64. The Mechanism of Neuritis in Vitamin B_1 Deficiency, 64. The Mechanism of Cardiovascular Failure in Vitamin B_1 Deficiency, 64. The Mechanism of Edema Formation in Vitamin B_1 Deficiency, 67. Vitamin B_1 Deficiency and the Gastro-intestinal Tract, 68. Vitamin B_1 Deficiency in Relation to Growth and Maintenance of Body Weight, 70. Vitamin B_1 Deficiency in Relation to Reproduction, Pregnancy, and Lactation, 70. Vitamin B_1 Deficiency in Relation to Infancy and Childhood, 71. The Relation of Vitamin B_1 to Carbohydrate Metabolism, 71. The Relation of Vitamin B_1 to Hematopoiesis, 72. Relation of Vitamin B_1 to the Other Vitamins and to the Endocrines, 73.

V. Pharmacology of Vitamin B_1 79

Pharmacological Effects, 79. Toxicology, 81.

VI. Prevention and Treatment 84

Need of Improved Dietaries, 84. Dietary Precautions, 85. Prophylactic Use of Vitamin B_1 Preparations, 85. Treatment, 86. Claims Allowed by American Medical Association, 86. General Principles of Treatment, 87. Method of Treatment, 88. Specific Treatment in the Adult and Infant, 88. Symptomatic Treatment in the Adult and Infant, 89. Treatment of Co-existent Diseases, 90. Available Preparations, 90. Need of Standardization of Labeling, 91.

VII. Human Diets in Relation to Beriberi 94

Cowgill's Analysis, 94. Reanalysis, 95. Thiamin-Calorie Ratio, 100. Thiamin-Carbohydrate Ratio, 100. Thiamin-Non-Fat-Calorie Ratio, 101. Other Approaches to Determination of Human Requirement, 102. Quantitative Statement of Human Requirement, 103. Milling of Grain, 103.

VIII. Occidental Diets 106

Adequacy of Thiamin in Occidental Diets, 106. Thiamin-Non-Fat-Calorie Ratios of Diets of Wage

CHAPTER PAGE

Earners in the United States, 107. Comparison of Thiamin Content of Oriental and Occidental Diets, 109. Classification of Foodstuffs, 109. Appraisal of Particular Diets, 110. Thiamin Content of Therapeutic Diets, 111. Thiamin Value of Average Servings, 112.

PART II

HISTORICAL AND EXPERIMENTAL

IX. DISCOVERY OF THE VITAMINS 123

Oriental Origin of Knowledge, 123. Studies in Japan, Java, Malay States and Philippine Islands, 124. Origin of Name, "Vitamine," 126. Discovery of Scurvy Vitamin, 127. The Fat Soluble Vitamins, 127. Chemical Dissimilarity of Vitamins, 128.

X. DIFFERENTIATION OF VITAMIN B 130

Thermostable Component, 130. Pellagra Vitamin, 131. Vitamin B_2 or Riboflavin, 132. Vitamin B_6, 132. Nicotinic Acid and Amide, 133. Summary of B Factors, 133. Nomenclature, 134. Origin of Name, "Thiamin," 134.

XI. ISOLATION OF THIAMIN 138

Extent of Experimental Studies, 138. Fuller's Earth as Adsorbent, 139. Jansen and Donath's Isolation, 139. Discovery of Sulfur in the Molecule, 140. Improvements in Yield, 140. A Useful Technique, 140.

XII. STRUCTURE AND SYNTHESIS OF THIAMIN 144

Structure, 144. Salient Chemical Features, 144. Elucidation of Structure, 146. Syntheses, 150.

XIII. REACTIONS, TESTS AND STANDARDS OF THIAMIN . . 158

Thermolability and Sensitivity to Alkali, 158. Destruction by Sulfite, 161. Oxidation-Reduction, 161. Action of Alkali, 162. Significance of *p*H, 164. Other Reactions, 165. Salts, 166. Tests for Identity and Purity, 167. Units, 168.

XIV. FUNCTIONAL GROUPS OF THE THIAMIN MOLECULE . 172

Thiamin Pyrophosphate or Cocarboxylase, 172. Thiochrome, 174. Synthesis of Thiochrome, 174. Analogues of Thiamin, 176.

XV. PYRIMIDINES ASSOCIATED WITH THIAMIN 180

Bioactivity of Thiamin Intermediates, 180. Biosynthesis of Thiamin from Intermediates, 180. Bioactivity of Variants of Pyrimidines Portion, 181. Synthetic Procedure, 183. List of Pyrimidines, 183.

CHAPTER PAGE

XVI. Thiazoles Related to Thiamin 191

Bioactivity of Related Thiazoles, 191. Functional Groups of Thiazole Portion, 192. Synthetic Procedures, 193. List of Related Thiazoles, 194.

XVII. Methods of Assay of Thiamin 197

Summary of Methods, 197. Comparative Merits, 198. Protective Methods, 200. Animal Growth Methods, 202. Coprophagy, 203. Weight Maintenance Methods, 206. Appetite and Weight Restoration Methods, 206. Curative Methods, 207. Bradycardia Test, 210. Units and Standards, 213. Chemical Methods of Assay, 216. Assay by Growth of Microorganisms, 220. Summary, 221.

XVIII. Distribution of Thiamin in Foods 226

General Considerations, 226. Animal Tissues, 228. Dairy Products, 229. Legumes, 229. Cereals, 229. Vegetables, 233. Fruits, 233. Miscellaneous, 234. Tables of Thiamin Values of Foods, 236.

XIX. Storage and Excretion 243

Thiamin Distribution in Organs, 243. Rate of Depletion, 244. Excretion in Feces and Urine, 250. Effect of High Levels of Intake on Storage and Excretion, 251. Summary, 253.

XX. Thiamin Requirement in Relation to Quantity of Food 256

Review of Literature, 256. Concept of Thiamin Calorie Ratio, 258. Induction of Polyneuritis by Forced Feeding, 259. Metabolic Rate and Thiamin Requirement, 261. Summary, 264.

XXI. Thiamin Requirement in Relation to Proportion of Carbohydrate, Fat and Protein 266

Early Experiments, 266. Thiamin Sparing Action of Fats, 266. Synthetic Fatty Esters, 268. Possible Role of Thiamin in Conversion of Carbohydrate to Fat, 274. High Liver Glycogen in Avitaminosis, 275. Effect of Protein Intake on Thiamin Requirement, 276. Summary, 277.

XXII. Thiamin Requirement in Relation to Special Circumstances 280

Growth on Graduated Doses of Thiamin, 280. Influence of Choice of Diet, 280. Influence of Choline, 283. Reproductive Function, 285. Thiamin in Relation to Appetite, 288. Thiamin in Special Relation to Growth, 290. Thiamin in Milk, 292. Thiamin in Relation to Lactation, 293. Thiamin and Intercurrent Disease, 295. Summary, 296.

CHAPTER PAGE

XXIII. Thiamin Requirement in Relation to Weight . 300

Cowgill's Experiments, 300. Reinterpretation, 300. Thiamin Requirement and Weight, 304. Weights of Japanese Sailors in Relation to Incidence of Beriberi, 306. Summary, 308.

XXIV. Thiamin Requirement in Relation to Species . . 309

Rising Proportional Requirement with Diminishing Size of Species, 309. Increasing Rapidity of Onset of Polyneuritis with Diminishing Size, 312. Approximate Uniformity of Thiamin-Calorie Ratio Required by all Species, 313. Summary, 314.

XXV. Disturbance of Glycogen Storage and Blood Sugar in Thiamin Deficiency 316

General Considerations, 316. Liver Glycogen in Avitaminosis, 318. High Liver Glycogen in Thiamin-Deficient Pigeons, 320. Conflicting Evidence, 322. Lajos' Experiments, 325. Blood Sugar, Insulin, Thiamin and Riboflavin, 328. Summary, 328.

XXVI. Thiamin in Intermediate Carbohydrate Metabolism 331

Non-Specificity of Thiamin in Relation to Special Functions, 331. Carbohydrate Metabolism Carried on in All Tissues, 332. Carbohydrate Metabolism as a Stepwise Process, 334. Enzyme Systems Involved, 335. Deficiency of Oxidases in Tissues, 337. Catatorulin Action of Vitamin B_1, 337. Occurrence of Pyruvic Acid in Relation to Nervous Disturbances, 340. Action of Thiamin Pyrophosphate as Cocarboxylase, 344. Combined Oxidative and Decarboxylating Effects, 345. Possible Synthesis of Fat from Pyruvic Acid, 346. Reconciliation of Divergent Findings, 349. Summary, 351.

XXVII. Concomitant Chemical Derangements . . . 356

Frequency of Multiple Deficiencies as a Complicating Factor, 356. Loss of Appetite, 357. Disturbances of Digestion, 358. Blood Chemistry, 360. Endocrine Functions, 360. Mineral Metabolism, 361. Glutathione, 362. Methyl Glyoxal, 363. Lipide Metabolism, 364. Summary, 365.

XXVIII. The General Role of Thiamin in Living Things 371

Thiamin and Other Nutrilites, 371. Thiamin and Yeast Growth, 373. Organisms Containing Thiamin: (*a*) Higher Plants, 375. (*b*) Fungi, 378. (*c*) Microorganisms, 378. Use of Thiamin by Organisms: (*a*) Higher Plants, 379. (*b*) Fungi, 381, (*c*)

CHAPTER PAGE

Microorganisms, 382. Thiamin Intermediates, 383. Substitutes for Thiamin Intermediates, 384. Thiamin and Insects, 385. Summary, 386.

XXIX. Recapitulation 391

Index of Names 397

Subject Index 405

PART I

VITAMIN B_1 (THIAMIN) IN THE PRACTICE OF MEDICINE

CHAPTER I

GENERAL CLINICAL CONSIDERATIONS

Toward the end of the nineteenth century, the concept arose that certain "accessory" food substances are essential for supporting life and maintaining health. These substances we now call vitamins. Their history is summarized in Chapters IX and X. As our knowledge of the function of the vitamins grew, it was realized that beriberi, sprue, rickets, pellagra, scurvy, and celiac disease develop when certain vitamins are not present in sufficient quantities in the diet. The necessity of proper balance among the vitamins was also recognized, but just what is the proper balance has not as yet been determined.

Today the importance of certain of the vitamins is not universally accepted by those concerned with problems of optimum health because of the relatively infrequent occurrence of the classical nutritional deficiency diseases. When the unmistakable forms of a disease are rare and the borderline examples uncommon, it is difficult for many to believe that the subclinical forms are so numerous and so important in the health of the general population. The diet of the American people is one of the best in the world, yet many Americans lack an optimum diet for growth and full vigor. Poverty, erroneous dietary habits, idiosyncrasies, and the presence of diseases are factors which result in a faulty diet for a considerable portion of the population. This accusation of fault in the American diet is made most often in connection with vitamins and minerals (1). The "refined" foods, commonly used, are poorer in vitamins than are natural foods, and the average person consuming large amounts of white flour, corn meal, polished rice, refined cereals, and sugar is more likely to develop a deficiency than is one who eats only unrefined foods.

A number of years ago McCollum (2) and McCarrison (3) pointed out that food deficiency in man is seldom limited to one particular factor. In the past few years it has been demonstrated that even "vitamin B" is composed of more than one "accessory" food substance. In 1936 R. A. Peters (4), in his communication to the Section of Nutrition at the Annual Meeting of the British Medical Association at Oxford, stated: "Research in many laboratories has shown that at least six entities compose the vitamin

B complex, which can be described as follows: vitamin B_1 (aneurin or torulin [thiamin; for a discussion of names see Chapter X, page 134]); vitamin B_2—lactoflavin and vitamin B_6 (rat antidermatitis); vitamin B_3 and B_5 (including the chicken antipellagra factor); vitamin B_4; human antipellagra (P.P. factor); dog black-tongue factor: Bios and growth factors for microorganisms." At the present time American investigators separate the vitamin B complex into vitamin B_1 and the vitamin B_2 complex. In the vitamin B_2 complex they distinguish riboflavin, vitamin B_6, and the "filtrate factor" of Jukes and Lepkovsky. It is likely that still other well-defined factors will be found in the near future (see Chapter X and particularly the Frontispiece).

The authors and many other investigators accept the point of view that in practically all states of malnutrition in human beings, the diet, because it is deficient in several factors, may eventually produce a complex clinical picture. It follows, then, that the physician will frequently recognize two or more distinct deficiency diseases in the same person (5, 6, 7, 8). These multiple deficiencies may arise not only from a defective dietary but also from abnormal utilization of the essential constituents of the diet. In human beings three factors operate to produce a dietary deficiency disease: 1. There may be an inadequate intake of essential food elements. 2. Absorption may be inadequate because of altered gastro-intestinal function (9, 10, 11). This alteration of function includes failure of the digestive processes, whether due to lack of proper enzymes (see Chapter XXVII) or to the excessive and rapid loss of food by vomiting and diarrhea. 3. The supply may be adequate and the absorption efficient, but a deficiency may arise because of an increased demand or as a result of some interference with storage or some failure of utilization by the cells. Pregnancy, lactation, growth, fever, exercise, insomnia, and hyperthyroidism increase the demand for at least some of the vitamins.

It is becoming more apparent that prolonged inadequacy of vitamins, either from dietary lack, failure of utilization, or increased demand, produces a variety of borderline states of ill health. These states are rarely well-defined and rarely appear uncomplicated. In human beings the contributory factors are usually complex and the environment cannot be controlled as in animal investigation. These factors explain, in part, the complexity of the resulting clinical picture seen in human beings with deficiency diseases. A person with a deficiency disease may suffer from any or all combinations of and from complete or partial deprivation of vitamins A, B_1, B_2 complex, C, and D, along with a lack of essential proteins and inorganic chemical substances. Clinicians are beginning to realize that the effects

of a persistent, slightly faulty diet may not be detectable for years. Careful questioning of patients with pellagra, beriberi, rickets, and scurvy shows that the early symptoms are general and extremely vague. The early symptoms of dietary deficiency diseases in human beings have been studied but little and demand the attention of clinicians. Some of the more important symptoms arising in the adult from various dietary deficiency diseases are presented in the following table, which is a slight modification of the one given by Haden (12):

SYSTEMS INVOLVED AND LESIONS OBSERVED IN DEFICIENCY DISEASE IN ADULTS

System	*Lesions Observed as Manifestation of Deficiency*
I. Skin and other epithelial tissues.	(1) Atrophy, (2) scaling, (3) dermatitis, (4) pigmentation, (5) ulceration, (6) cornification.
II. Nervous system	A. Neuritis: (1) pain, (2) paresthesia, (3) weakness, (4) paralysis.
	B. Degeneration of spinal cord: (1) lateral column, (2) posterior column.
	C. Cerebral: (1) mental disturbances.
	D. Disturbance of function: (1) tetany.
III. Alimentary tract	(1) Anorexia, (2) stomatitis, (3) glossitis, (4) atrophy of tongue, (5) achlorhydria, (6) loss of specific ferments, (7) diarrhea, (8) loss of tone of gastrointestinal tract, (9) ulceration of intestine.
IV. Hematopoietic system	(1) Macrocytic anemia, (2) hypochromic anemia, (3) microcytic anemia.
V. Cardiovascular system	(1) Hemorrhage, (2) easy bruising, (3) edema, (4) nutritional heart disease.

Vitamin B_1 deficiency frequently occurs during infancy and childhood (13, 14, 15). The susceptibility of infants to vitamin B_1 deficiency is of such magnitude that infantile beriberi occurs much more frequently than does adult beriberi. It is common knowledge that nursing mothers may be free from any serious symptoms of beriberi, and yet their infants develop fatal beri-

beri. A detailed description of symptoms will be found under the section on infantile beriberi (see Chapter II).

The authors and other investigators have frequently observed infants, children and adults who show some clinical evidence of vitamin B_1 [thiamin] deficiency associated with one or several of the following objective changes resulting from deficiencies of other essential nutritional factors:

1. **Buccal, lingual, and mucous membrane abnormalities,** as found in sprue, pellagra, pernicious anemia, or other related diseases. The earliest and most common manifestation is a reddened appearance of the papillae over the anterior third and tip of the tongue. This process may extend to involve the adjacent portion of the tongue and may be followed by diffuse glossitis associated with fiery redness and sometimes with pain. Aphthous ulcers may appear over the tongue and buccal mucosa, and the fiery redness may likewise extend to the mucous membranes of the mouth. This is the appearance of the stomatitis in cases of classical pellagra (16). Where the papillae are atrophic and there is little or no fiery redness of the tongue, the picture is more like that seen in sprue or pernicious anemia (17, 18, 19). Most investigators in the field of deficiency diseases consider all of the above changes to be nutritional in origin.

2. **Abnormal conditions of the skin** (20, 21). Such conditions are usually preceded or accompanied by other clinical manifestations of a deficiency disease. The most common dermal change associated with malnutrition is an abnormally dry and scaly texture, especially of the skin over the extremities. This dryness may be associated with hyperkeratosis. Following the administration of an adequate diet and improvement in the patient, the skin will again appear normal. Other dermal changes which may be present, though less frequently, are pigmentation and ulceration of the skin, and mild or extensive dermatitis characteristic of pellagra. By the time such lesions are diagnosed as pellagrous, other manifestations of the disease usually have already appeared.

3. **Microcytic or macrocytic anemia.** About 50 per cent of the patients seen in the Cincinnati General Hospital, which admits severe, acute, and chronic diseases, have hemoglobin values below 70 per cent (Sahli). The anemia in the great majority of these patients can be cured by the administration of liver or stomach preparations, yeast, or iron, or by the administration of several of these substances.

4. **Edema** associated with malnutrition and without evidence of cardiac or renal insufficiency or hypoproteinemia (22).

5. **Reduction of inorganic minerals in the blood.**

6. **Night blindness** due to vitamin A deficiency. The association, however, between clinical night blindness and vitamin A

deficiency as revealed by the biophotometric test has not as yet been fully demonstrated (23, 24, 25).

Since the diet of many people is low in vitamin B_1, and since man does not store this vitamin to any significant degree, manifestations of its lack may occur alone. That is to say, not all vitamin B_1 deficiency states are part of a multiple deficiency syndrome (26). Human beings show ill health on a vitamin B_1 deficient diet long before recognizable symptoms of beriberi appear (27, 28). It can readily be seen that it is impossible to recognize the beginning of vitamin B_1 deficiency, as there is neither a simple objective test for the lack of this substance nor are there pathological effects which are specific or easily identified. Nevertheless, careful study has convinced most students of the disease that subclinical forms of vitamin B_1 deficiency occur frequently.

A lack of vitamin B_1 is particularly common in association with the following: 1. *Poverty.* Lack of knowledge as well as the lack of money is of fundamental importance in the association of poverty with vitamin B_1 deficiency. If sufficient amounts of a well-balanced diet are eaten, vitamin B_1 deficiency usually does not develop. Education of the poor in correct dietary habits so that they will buy inexpensive, protective foods or obtain protective substances such as yeast, wheat germ, and tikitiki (rice polish) extract through relief agencies will greatly decrease the incidence of vitamin B_1 deficiency among the indigent. 2. *Improper dietary habits.* It is a popular fallacy to believe that our diets are adequate merely because we are accustomed to them. Many people undoubtedly live on a diet of narrow range, usually high in fats and carbohydrates and low in protein, minerals, and vitamins. Because of custom or preconceived ideas as to what foods are good for them, or because of dependence upon appetite as a guide in the proper selection of foods, many people are likely to choose a poorly balanced diet. 3. *Organic disease.* Organic disease often predisposes to the development of a deficiency of vitamin B_1 (29, 30). Certain diseases are associated with loss of appetite, and accordingly the person may ingest less. In other diseases the absorption may be reduced or the general requirement increased. These factors may exist simultaneously. Generally speaking, in most diseases accompanied by disturbances of the gastro-intestinal tract, some kind of restricted diet is prescribed by the physician or is voluntarily observed by the patient. A well-balanced diet is not generally considered in the patient's search for relief or in the physician's zeal to prevent discomfort. Hence, some of the strictly curative diets are often deficient in vitamin B_1 (see Chapter VIII). The proper evaluation of the symptomatology of vitamin B_1 deficiency is particularly difficult in the presence of another disease, as the deficiency and the

disease often operate together and either or both may produce the symptoms in question.

The prodomal symptoms of vitamin B_1 deficiency are loss of appetite, weight, and strength; muscle cramps; diarrhea, abdominal pain; palpitation; dyspnea; edema; burning sensations in various parts of the body; vertigo; headache; cessation of menstruation; numbness and tingling; nervousness; depression; irritability; distractibility; apprehension and forgetfulness.

If deprivation of vitamin B_1 is sufficiently prolonged, the majority of these symptoms group themselves as arising from the alimentary tract, the cardiovascular system, and the nervous system.

1. **The alimentary tract.** Undoubtedly vitamin B_1 is essential for the normal functioning of the gastro-intestinal tract. In some cases its lack produces anorexia; in others, the motility is abnormal. There are no specific lesions, however, that are clearly understood at the present time. The gastro-intestinal tract may be altered in such a way that vitamin B_1 is not absorbed.

2. **The cardiovascular system.** The cardiovascular system is strikingly affected in some persons with a vitamin B_1 deficiency. The early symptoms of palpitation and dyspnea are the same as those experienced from any other type of heart disease. In certain patients there may be acute cardiac failure (see beriberi heart), and in still others, heart failure may or may not be associated with edema and serous effusions. The tendency toward edema and serous effusions cannot be fully explained at the present time, but the underlying causes of edema in general are well summarized in the following table by Landis (31).

Factors Favoring Edema Formation	*Clinical Examples*
A. *Primary:*	
1. Elevated capillary pressure	1. (*a*) Venous obstruction (*b*) Thrombophlebitis (*c*) Cardiac edema with venous congestion
2. Lowered colloid osmotic pressure	2. (*a*) Nutritional edema (*b*) Nephrotic edema (*c*) Cardiac edema, late stages with malnutrition
3. Damage to capillary wall	3. (*a*) Inflammatory edema (*b*) Nephritic edema (*c*) Cardiac edema(?), chronic anoxemia
4. Lymphatic obstruction	4. (*a*) Lymphedema (*b*) Cardiac edema with venous congestion

B. *Contributory:*	
5. Low tissue pressure	5. Edema of periorbital tissues and genitalia
6. High salt intake	6. Increases edema if water is available
7. High fluid intake	7. Increases edema if salt is available
8. Warm environment	8. (*a*) Heat edema (*b*) Increases all types of edema
9. Disturbed innervation	9. (*a*) Tropho-edema (*b*) Unilateral edema in hemiplegia

3. **The nervous system.** While there is no definite evidence that symptoms arising from the brain and spinal cord result from vitamin B_1 deficiency in human beings, it is generally agreed that the peripheral neuritis of beriberi is caused by a lack of this vitamin.

Peripheral nerves. Although arbitrary in many respects and obviously imperfect in others, the best clinical classification of peripheral neuritis, from the standpoint of etiology, is that of Cobb and Coggeshall (32) (see Table I).

As seen in the above table, these authors divide peripheral neuritis into local and general and in that way separate the unilateral disorders attacking only one nerve from the large group of systematic disorders which cause bilateral polyneuritis.

Localized neuritis. This type of neuritis usually occurs in ambulatory patients, and the physician can often demonstrate the mechanical or infectious etiological agent by the usual clinical methods. The symptoms often respond promptly to proper medical and surgical therapy. Relief of various types of localized neuritis following the administration of crystalline vitamin B_1 has been reported by various workers (33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47). The reports on the various types of localized neuritis are probably one-sided, since physicians who obtain negative results are less prone to report them. The number of patients in the reported series is often small, and in general the studies are not adequately controlled; hence, the results need confirmation. No conclusive reports have as yet appeared in the literature concerning the use of vitamin B_1 in the prevention of localized neuritis. Now that vitamin B_1 crystals are available, it is expected that controlled observations will be made on these points.

Generalized polyneuritis. The *virus diseases* listed in Column I are often accompanied by peripheral neuritis as a late complication. No crucial observations have been made to de-

TABLE I

PRINCIPAL CAUSES OF NEURITIS

NEURITIS

GENERALIZED POLYNEURITIS

Virus	Bacteriotoxic	Deficiency or Metabolism	Chemical
Measles	Focal infections	Pellagra	Mercury
Smallpox	"Rheumatism"	Pernicious anemia	Lead
Chickenpox	Erysipelas	Sprue	Silver
Parotitis	Scarlet fever	Beriberi	Arsenic
Herpes	Rheumatic fever	"Alcoholic neuritis"	Phosphorus
"Acute febrile"	Chorea	"Korsakoff's psychosis"	Methyl alcohol
"Acute infective"	Septicemia	Pernicious vomiting	Ethyl alcohol
"Landry's"	Puerperal fever	Hunger edema	Ethyl iodide
Poliomyelitis	Gonorrhea	Pregnancy	Trichlorethylene
Encephalomyelitis	Meningitis	Chronic colitis	Carbon tetrachloride
Epidemic (lethargic) encephalitis	Diphtheria	Cancer with cachexia	Trinitrotoluene
Erythro-edema	Typhoid fever	Tuberculosis with cachexia	Dinitrobenzene
Acute rabic myelitis	Paratyphoid fever	Senility with cachexia	Triorthocresyl phosphate
	Typhus fever	Diabetes	Aniline
	Influenza	Myxedema	Sulphonethylmethane barbital, etc.
	Pneumonia	Hematoporphyrinuria	Chloral, Chlorbutanol
	Malaria	"Recurrent polyneuritis"	Carbon monoxide
	Relapsing fever	"Chronic progressive polyneuritis"	Carbon bisulphide
	Serum sickness	Chronic bacillary dysentery	Thallium
	Acute enteric fever		Sulphur
	Syphilis		Emetin
			Gold
			Bismuth

LOCALIZED NEURITIS

Mechanical	Infectious
Pressure	Diphtheria
tumor	Tetanus
edema	Streptococci
arthritis	Leprosy
fibrosis	
Trauma	
Saturday night paralysis	
Volkman contracture	
Meralgia paresthetica	

termine whether faulty nutrition plays a role in the development of the neuritis accompanying these diseases. The experimental evidence available tends to show that vitamin B_1 does not play any direct specific role in counteracting these infectious agents.

In the column under *bacteriotoxic* is a long list of infectious diseases which may be complicated by peripheral neuritis. The diseases in this group often interfere with proper nutrition and thus predispose the patient to the development of vitamin B_1 deficiency. Many of these patients with infection also have an increased requirement for essential nutritive substances, and in some instances the ingested food is not sufficient to meet this greater demand. In typhoid fever, for example, the requirement for vitamin B_1 is raised because of the high temperature, and the supply may be diminished because of loss of appetite or excessive diarrhea. The incidence of peripheral neuritis in patients with typhoid fever was high a few decades ago, when an effort was made to restrict the diets of such patients. The great decrease in the incidence of peripheral neuritis in typhoid fever in recent years is probably due to the substitution of adequate diets in the treatment of that disease. We have no information concerning the role of vitamin B_1 deficiency in the development of any of these neuritides, but a number of investigators (33, 43, 46, 47) have reported that the administration of vitamin B_1 oftentimes relieves the symptoms of peripheral neuritis. It is known that the peripheral neuritis in certain persons with infectious diseases disappears following the administration of a well-balanced diet; hence, it seems advisable to consider carefully the diets of all persons with infectious diseases complicated by peripheral neuritis.

In the fourth column can be seen a long list of *chemical substances* which are known to cause peripheral neuritis. Toxicologists and neurologists agree that not every person exposed to the same chemical agent responds in an identical manner. There is definite clinical evidence showing the predilection of some of these substances for special portions of the nervous system. Aring, Zschiesche, and Spies (48) tried without success to alter the course of two cases of triorthocresyl phosphate poisoning by means of large amounts of crystalline vitamin B_1. One of the patients received 20 milligrams daily for a period of one month, and the other received 500 milligrams daily for the same length of time. It is theoretically possible that the ingestion of large amounts of vitamin B_1 crystals before the ingestion of the Jamaica ginger might act as a protective agent. It has been reported that the administration of vitamin B_1 causes relief of the symptoms of the polyneuritis following exposure to toxic agents such as nicotine, lead, thallium, arsenic, and mercury (34, 46, 49, 50). As yet, however, there is no conclusive evidence

as to whether lack of vitamin B_1 plays a definite role in the development of any of the "chemical neuritides."

Under Column III one sees a long list of diseases which show the conspicuous relationship of the lack of proper nutrition to the development of neuritis. This list is composed chiefly of *metabolic and deficiency diseases.* These diseases often have a number of clinical features in common. In the scope of this book we are concerned chiefly with the neuritic manifestations which may be common to patients having these diseases. Evidence is accumulating rapidly which shows that many of the polyneuritic symptoms listed under various syndromes are clinical variations of one large etiological group. Since vitamin B_1 will prevent and usually relieve the clinical manifestations of the polyneuritis of beriberi, a known dietary deficiency disease, and since it is essential for the integrity of the nervous tissue in general, it seems likely that its absence may play a very important part in the development of neuritis in a number of these diseases. In fact, most of the evidence points to the fact that it is a useful therapeutic agent in the treatment of alcoholic neuritis with or without pellagra, polyneuritis of pregnancy, and polyneuritis of diabetes (see Chapter III). Most of the other diseases in this column may be related to disturbances of nutrition, and it may well be that a lack of vitamin B_1 plays a role in the production of neuritic manifestations accompanying these other diseases. These diseases of metabolism are often associated with loss of appetite and malabsorption which, in themselves, may lead to a deficiency of some specific factor or factors. These manifestations are extremely variable from a clinical standpoint. For example, if a physician studies a large group of chronic alcoholic addicts with malnutrition, he will find some with no symptoms, others with symptoms of beriberi, and still others with neurological, dermal, and gastro-intestinal changes characteristic of pellagra. Still others will show hematological and neurological changes similar to those of sprue and of pernicious anemia. Some of the persons with chronic alcoholic addiction develop Korsakoff's syndrome. This clinical syndrome is characterized by a peripheral neuritis identical with that of beriberi and by a psychosis often resembling that of pellagra.

Before going into a clinical discussion of beriberi and some of the other related diseases often associated with peripheral neuritis or edema, it is well to realize that as the etiological agents are better understood, the present clinical classifications of the various syndromes will give way to a classification based entirely on the etiology.

BIBLIOGRAPHY

1. Tisdall, F. F.: Inadequacy of Present Dietary Standards. Can. Med. Asso. J. *33*, 624, 1935.
2. McCollum, E. V.: The Relation of Diet to the Cause and Treatment of Pellagra. Endocrinology and Metabolism. D. Appleton Company, New York, p. 911, 1932.
3. McCarrison, R.: The Pathogenesis of Deficiency Disease. Indian J. M. Research *6*, 275; cont. *6*, 550, 1919.
4. Peters, R. A.: The Vitamin B Complex. Brit. Med. J. *2*, 903, 1936.
5. Minot, G. R.: Some Fundamental Clinical Aspects of Deficiencies. Ann. Internal Med. *3*, 216, 1929.
6. McLester, J. S.: Clinical Syndromes That Include Achlorhydria. J.A.M.A. *95*, 719, 1930.
7. Keefer, C. S.: Some Clinical Aspects of Deficiency Diseases. New England J. Med. *205*, 1086, 1931.
8. McCann, W. S.: Multiple Dietary Deficiencies in Pernicious Anemia. Tr. Asso. Am. Physicians *43*, 106, 1928.
9. Strauss, M. B.: The Role of the Gastro-intestinal Tract in Conditioning Deficiency Disease. J.A.M.A *103*, 1, 1934.
10. Mackie, T. T., and Pound, R.E.: Changes in the Gastro-intestinal Tract in Deficiency States, with Special Reference to the Small Intestine: A Roentgenologic and Clinical Study of Forty Cases. J.A.M.A. *104*, 613, 1935.
11. Hartsock, C. L.: A Consideration of the Deficiency Factor in Diseases of the Gastro-intestinal Tract. Cleveland Clinic Quart. *3*, 161, 1936.
12. Haden, R. L.: Multiple Specific Nutritional Deficiency Disease in the Adult. J.A.M.A. *106*, 261, 1936.
13. Blackfan, K. D., and Wolbach, S. B.: Vitamin A Deficiency in Infants: A Clinical and Pathological Study. J. Pediat. *3*, 679, 1933.
14. Hoobler, R. R.: Use of Vitamin B in Diets of Infants; Further Observations. J.A.M.A. *96*, 675, 1931.
15. Griffith, J. P. C., and Mitchell, A. G.: The Diseases of Infants and Children, 2d. Ed. W. B. Saunders Company, Philadelphia, 1937.
16. Spies, T. D.: Pellagra. Cecil's Textbook of Medicine. 4th Ed. W. B. Saunders Company, Philadelphia, 1937.
17. Reed, A. C.: Sprue: A Clinical Summary. Am. J. Trop. Med. *16*, 499, 1936.
18. Musser, J. H.: Sprue. Internal Medicine; Its Theory and Practice. Lea and Febiger, Philadelphia, p. 357, 1932.
19. Hanes, F. M.: Sprue. Cecil's Textbook of Medicine, 4th Ed. W. B. Saunders Company, Philadelphia, 1937.
20. Youmans, J. B.: The Present Status of Vitamin Deficiencies in Practice. J.A.M.A. *108*, 15, 1937.

21. Loewenthal, L. J. A.: A New Cutaneous Manifestation in the Syndrome of Vitamin A Deficiency. Arch. Dermat. and Syph. *28*, 700, 1933.
22. Elsom, K. O.: Experimental Study of Clinical Vitamin B Deficiency. J. Clin. Investigation *14*, 40, 1935.
23. Jeans, P. C., and Zentmire, Z.: A Clinical Method for Determining Moderate Degrees of Vitamin A Deficiency. J.A.M.A. *102*, 892, 1934.
24. Jeghers, H.: The Degree and Prevalence of Vitamin A Deficiency in Adults with a Note on its Experimental Production in Human Beings. J.A.M.A. *109*, 756, 1937.
25. Frandsen, H.: Investigation of Hemeralopia as Evidence of Minor Degrees of Vitamin A Deficiency. Nutrition Abst. & Rev. *4*, 621, 1935.
26. Cowgill, G. R.: Vitamin B_1 in Relation to the Clinic. J.A.M.A. *98*, 2282, 1932.
27. Spies, T. D.: Unpublished observations.
28. Jolliffe, N.: Personal communication.
29. Wilder, R. M., and Wilbur, D. L.: Diseases of Metabolism and Nutrition. Arch. Internal Med. *59*, 512, 1937.
30. Wilbur, D. L.: Vitamin Deficiency Diseases: Their Diagnosis and Treatment. M. Clin. North America *21*, 737, 1937.
31. Landis, E. M.: The Mechanism of Edema Formation. Modern Concepts of Cardiovascular Disease. Vol. 4, No. 11, November, 1935.
32. Cobb, S., and Coggeshall, H. C.: Neuritis. J.A.M.A. *103*, 1608, 1934.
33. Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1. Experimental and Clinical Observations. J.A.M.A. *105*, 1580, 1935.
34. Ruschke: Versuche mit Betaxin als Antineuritikum. Deutsch. med. Wochschr. *62*, 181, 1936.
35. Hofer von Lobenstein, M.: Vitamin B_1 Avitaminose, ihre Beziehung zu Neuritischen Affektionen und deren Behandlung mit Betaxin. Münch. med. Wochschr. *83*, 510, 1936.
36. Peters, B. A., and Cunningham, R. N.: Vitamin B_1 and Diphtheria. Lancet, I, 563, 1937.
37. Knapp, E.: Betaxinerfolg bei Postdiphtherischer Zwerchfellähmung. Kinderärztl. Praxis *8*, 198, 1937.
38. Bennholdt-Thomsen, C.: Betexinerfolg bei Postdiphtherischer Zwerchfellähmung? Kinderärztl. Praxis *7*, 545, 1936.
39. Kühnau: Improvement Following Vitamin B_1 Therapy in Cases of Herpes Zoster. E. Merck's Jahresber. *49*, 49, 1935.
40. Schwochow, P.: Erfahrungen mit Betaxin (Vitamin B_1-Präparat) in der Behandlung von Neuritiden und Polyneuritiden aller Art. Münch. med. Wochschr. *84*, 98, 1937.

41. Hesse, E.: Die Neuritis Cruralis und ihre Behandlung mit Vitamin B_1. Münch. med. Wochschr. *83*, 356, 1936.
42. Sciclounoff, F., and Broccard, R.: La Vitamin B_1 dans le Traitement des Polynévrites. Schweiz. med. Wochschr. *66*, 985, 1936.
43. Neumann, H.: Erfahrungen mit Betaxin (Vitamin B_1-Präparat) in der Neurologie. Münch. med. Wochschr. *82*, 1959, 1935.
44. Shastid, T. H.: Optic Neuritis and Vitamin B. Am. J. Ophthalmol. *12*, 903, 1929.
45. Pflimlin, R.: B-Avitaminosen des Auges. Schweiz. med. Wochschr. *67*, 422, 1937.
46. Heiman, M.: Zur Vitaminbehandlung der Nervenkrankheiten; Klinik und Therapie des Vitamin B_1. Klin. Wochschr. *16*, 1076, 1937.
47. Stepp, W.: Vitaminmangel als Ursache und Folge von Magen-Darmerkrankungen. Münch. med. Wochschr. *83*, 1119, 1936.
48. Aring, C., Zschiesche, E., and Spies, T. D.: Unpublished observations.
49. Fellinger, K.: Klinik und Therapie der Bleivergiftung und Quecksilberschäden. Wien. klin. Wochschr. *50*, 1542, 1936.
50. Gallowitsch, P.: Betaxin (Vitamin B_1) bei Arsen-Polyneuritis. Münch. med. Wochschr. *84*, 1138, 1937.

CHAPTER II

BERIBERI AND CLOSELY RELATED OR IDENTICAL DISEASES

BERIBERI—SHIP BERIBERI—EPIDEMIC DROPSY—HUNGER EDEMA—SPORADIC CASES IN ASSOCIATION WITH MALNUTRITION

BERIBERI

SYNONYMS

Polyneuritis endemica; barbiers (France); loempoe (Java); kakke (Japan and China); taon (Philippines); maladie des sucreries (French Antilles); maladie des jambes (Louisiana); hinchazon (Cuba); inchacao or pernieras (Brazil).

DEFINITION

Beriberi is a disease caused by a prolonged deficiency of the antineuritic vitamin, B_1. It is non-contagious and non-hereditary and may occur at any age in either sex of any race. In the adult it is characterized by multiple neuritis, muscular atrophy, cardiovascular changes, serous effusions, and generalized edema. These manifestations vary so greatly in number, severity, and order of appearance from one patient to another that beriberi has come to be classified into three types: 1. *Dry beriberi,* when the predominating symptom is a multiple peripheral neuritis. 2. *Wet beriberi,* when serous effusions and edema are the outstanding features. 3. *Cardiac, pernicious, or acute beriberi,* when fulminating cardiovascular changes are present. Observations on a large series of persons with beriberi show that individual cases frequently have findings characteristic of more than one of the above types. Such cases are often called *mixed beriberi.*

Like the adult, the infant develops beriberi when there is a deficiency of vitamin B_1 in the diet. Infantile beriberi is nearly always an acute disease with a sudden onset, and is characterized particularly by acute cardiac enlargement, dilatation, and, if untreated, sudden death.

Incidence

Beriberi occurs endemically where polished rice and other refined cereals, deficient in vitamin B_1, form the major portion of the diet. It is particularly prevalent in Japan, southern China, the Malay Peninsula, East Indies, the Philippines, and southern India. It has been observed frequently in Brazil, the West Indies, Australia, and on the east and west coasts of Africa. The disease occurs sporadically and in small outbreaks on shipboard, among government troops, and in prisons, insane asylums, and other institutions throughout the world.

History

According to Macgowan (1), in his *History of China,* beriberi was recognized by the Chinese as early as 2697 B.C., but it was unknown to physicians of the western world until 1645 A.D. In that year Jacobus Bontius (2) called attention to the presence of dry beriberi in Java. Over one hundred and fifty years were to pass before Rogers (3) described the wet form. Wet beriberi and dry beriberi were considered two separate diseases until 1835, when Malcolmson (4) observed that cases which began as wet beriberi often became transformed into dry beriberi after sudden diuresis and that the dry type often developed edema. From these observations he concluded that the wet and dry forms are two types of the same disease, an opinion which is now almost universally accepted. In 1898 infantile beriberi in nursing infants was described for the first time by Hirota (5).

Since the middle of the nineteenth century, studies have been directed particularly toward determining the cause of beriberi. Following the discovery that a deficiency of vitamin B_1 plays an essential if not complete role in the development of this disease, investigators devised and successfully applied dietary methods of prevention and treatment. In 1927 Jansen and Donath (6) reported the isolation of the antineuritic vitamin, B_1. Williams, Waterman, and Keresztesy (7), a few years later, were able to obtain it in relatively large quantities and eventually were able to synthesize it (8) (see Chapter XII). Although crystalline vitamin B_1 has, thus far, been administered to only a small number of persons with beriberi, the results obtained indicate that this substance is the specific antiberiberic factor. Hermano and Eubanas (9) treated ten adults and two infants with intramuscular injections of crystalline vitamin B_1 and concluded that this substance gave very promising indications of curing beriberi. Hashimoto (10) and Murakoshi (11) each treated successfully one case of beriberi with cardiac involvement

by means of crystalline vitamin B_1. The acute cardiac case reported by Hashimoto had severe myocardial damage as evidenced by electrocardiography. Following intravenous injections of crystalline vitamin B_1 there was prompt recovery and a return of the electrocardiogram to normal. It is hoped that within the next few years careful studies will be done on large series of cases in order that the effectiveness of crystalline vitamin B_1 in the treatment of the typical case of beriberi may be fully evaluated.

Etiology

An enormous amount of effort has been directed toward determining the cause of beriberi, with the result that many theories have been advanced only to be discarded for lack of evidence in their support. The two prevailing theories are: 1. Beriberi is an infection in which diet plays a more or less important role (12, 13, 14, 15). 2. It is a dietary deficiency disease. Recent studies have given little support to the theory of infectious origin, whereas the results of clinical and experimental studies have shown that beriberi develops following a faulty food supply or an alteration in metabolism (16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29). In most instances there is a relationship between the ingestion of an unbalanced diet, rich in decorticated cereals, and the development of the disease (see Chapter VII). Such diets are deficient in thiamin, but a final decision has not been reached as to whether the lack of thiamin is the sole cause of the syndrome, nor do we know the exact role which thiamin plays. The clinical manifestations of beriberi are so varied that more than one etiological, chemical substance may be responsible for the development of the syndrome. It is possible that a deficiency of thiamin may arise from one or several of the following: (*a*) a deficiency in the diet, (*b*) failure of absorption, (*c*) deficient storage, (*d*) failure of utilization, (*e*) increased requirement.

Apparently man, as well as experimental animals, is unable to synthesize thiamin, nor can he store it to any great extent. Thus he is particularly susceptible to the development of beriberi. It follows, then, that a person with severe beriberi undoubtedly has a significant depletion of his stores of thiamin. It is not known, however, how long the depletion of vitamin B_1 in man must exist before the symptoms of beriberi appear. Clinical studies show that the depletion period is extremely variable, ranging perhaps from a few weeks to months or even years (23, 24, 30, 31, 32, 33, 34, 35, 36, 37). It is obvious that from the state of complete saturation of vitamin B_1 to one of active beriberi, there must be a broad range in which the person is free from symptoms and yet subsists on a low intake of thiamin.

Although beriberi appears only in the absence of vitamin B_1, certain predisposing and precipitating factors play a definite role in the development of this clinical syndrome. Important among these factors are: poverty (20, 38, 39); increased physical exercise, hyperthyroidism, fevers, and other conditions which are accompanied by an increased metabolic rate (39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49); pregnancy and lactation (43, 50, 51, 52, 53, 54); fatigue (39, 42); food fads and dietary idiosyncrasies (55); and digestive disturbances and chronic debilitating diseases which cause improper ingestion, assimilation, or utilization of food (39, 43, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74).

However varied opinions may be in regard to the etiology of beriberi, it seems clear, from clinical and experimental studies, that the disease can be prevented and usually cured by the use of foods rich in vitamin B_1 or by supplements of thiamin (38, 75). Much of the present confusion over the relationship between the various symptoms of beriberi and between beriberi and other diseases should be explained within the next few years, as thiamin in pure form is now available for clinical studies.

Signs and Symptoms of Clinical Beriberi

In the infant, beriberi is usually an acute disease; in the adult, it is usually chronic.

Infantile type. The clinical picture of infantile beriberi is characterized by a sudden onset associated with rigidity of the body, whining, constipation, diminished urinary excretion, weakness, edema, cardiac enlargement, cyanosis, and a rapid and irregular pulse (76, 77, 78, 79, 80). If the infant is not cured promptly by means of treatment, he dies suddenly.

Adult type. In contrast to beriberi in the infant, the onset in the adult, except in cases of acute cardiac beriberi, is usually insidious, and the symptoms are vague and ill-defined. Loss of strength is one of the earliest subjective manifestations of the disease. The sole complaint for weeks may be fatigue and sensations of heaviness and stiffness of the legs, and perhaps inability to walk long distances. Soon after the appearance of these symptoms, a person developing beriberi often notices headache, insomnia, nervousness, dizziness, dyspnea, loss of appetite, dyspepsia, tachycardia, and tenderness of the calf muscles. After a variable period of time the major symptoms of beriberi can be divided into three groups, corresponding to the predominating pathological alteration: *a*. Symptoms referable to involvement of the nervous system produce a clinical picture which is called *dry beriberi*. *b*. Symptoms arising from and associated with generalized edema and serous effusions produce a clinical picture

which has been called *wet beriberi. c.* Acute symptoms depending upon cardiac involvement and including passive congestion of the viscera have been termed *acute pernicious beriberi.* Cases of beriberi having the characteristic symptoms of more than one of the above types are known as *mixed beriberi.*

The following table of Platt and Lu (81) attempts to correlate the major symptoms of beriberi with the degree of vitamin B_1 deficiency. This table is important in emphasizing the chief symptoms of clinical beriberi, but in the experience of the authors, any attempt to correlate the clinical findings with the degree of vitamin B_1 deficiency is much more difficult than would be suggested by the arbitrary symbols used by Platt and Lu.

BERIBERI: THE ASSESSMENT OF SYMPTOMS IN HUMAN BEINGS IN TERMS OF VITAMIN B_1 DEFICIENCY

	Vitamin B_1			
Clinical Symptoms	+	++	+++	++++
Neuritis usually of legs with altered (usually absent) knee- and ankle-jerks				
Paraesthesiae				
Weakness				
Oedema				
Enlarged heart				
Tachycardia (with embryocardia)				
Cyanosis				
Dyspnoea				
Cardiac decompensation				
Epigastric distress				
Prostration and restlessness				

Whether mild or severe, each case of beriberi presents individual variations in the symptoms of peripheral neuritis, cardiac insufficiency, and edema. Clinical studies of a group of patients with beriberi show that the number of symptoms present, their order of appearance, and their degrees of severity are extremely variable (20, 38, 39). For example, a patient with predominating cardiac symptoms may develop multiple peripheral neuritis, and conversely, a patient with peripheral neuritis may suddenly develop cardiorespiratory symptoms. Serous effusions and edema may precede, accompany, or follow the cardiac or nervous symptoms.

Nervous system. The major clinical manifestation is an ascending, symmetrical, peripheral neuritis. The initial symptoms are weakness and cramps of the legs, often followed by burning of the ankles and the soles and dorsal surfaces of the feet. The Achilles and patellar reflexes become increased, later diminished,

and finally they disappear. The muscles of the feet and calves, and finally the extensors and flexors of the thigh, are involved. By the time the thigh muscles are weak, toe and foot drop can usually be demonstrated. These neurological changes are associated with tenderness in the affected muscles, particularly the calf muscles, and with sharply defined anesthesia and numbness. Atrophy of the muscles and skin soon follows involvement of the nerve. As a rule, the upper extremities are not involved to any great degree until the leg symptoms have become pronounced. In the most severe cases, the process may extend until the muscles of the trunk and diaphragm are involved. Patients with such involvement are prone to have muscular contractures, ataxia, and lack of co-ordination. Sphincter control is usually maintained until the late stages. Beriberi strikingly affects the vagi. The disease, as a rule, is not accompanied by severe mental symptoms. Symptoms arising from neural degeneration may subside slowly in the adults who recover. In the severe case many months may pass before there is restoration of function, if, indeed, complete restoration does occur.

Cardio-respiratory symptoms. The symptoms of acute cardiac beriberi closely resemble those of infantile beriberi. Patients with predominating cardiac symptoms usually die suddenly, and often without a history of prodromal symptoms. At any time during the course of the disease, and often without warning to the physician, symptoms of acute cardiac failure may suddenly appear. This cardiac failure is characterized by paroxysmal onset of precordial pain, dyspnea, cyanosis, increased venous pressure, tachycardia, murmurs, and a small, thready pulse. Transient elevation of the systolic and diastolic blood pressure and electrocardiographic changes are frequently described (see Chapter IV). Examination of the heart shows striking enlargement, especially to the right, associated with pulmonary congestion, cyanosis, and vasomotor collapse. Weiss and Wilkins (82) recently reported thirty-five instances of "beriberi heart" in Boston, Massachusetts. In contrast to patients with so-called Oriental beriberi, the majority of their cases did not exhibit a pure right-sided failure of circulation. In fact, some of them showed pulmonary engorgement, dyspnea, orthopnea, and other manifestations of left-sided failure. Weiss and Wilkins are of the opinion that "beriberi heart" occurring in Boston is etiologically similar to beriberi heart of the Orient. Goodhart and Jolliffe (83) believe that beriberi in all of its manifestations is found in chronic alcoholic addicts.

Edema and other symptoms. Edema is the most conspicuous feature of many cases, and it may conceal underlying muscular atrophy. The edema begins in the feet and legs, and gradually extends up the body. Ascites, hydrothorax and hydropericardium

are often associated with the generalized edema. Following adequate therapy, the edema subsides rapidly, and atrophy of the muscles may be seen. Gastro-intestinal symptoms consisting of anorexia, diarrhea, and vomiting may be present, with or without edema. The volume of urine is diminished during the time the person is developing edema. Apparently the edema occurs without significant renal changes. Aphonia occurs with considerable frequency both in infantile beriberi and in chronic adult cases. A moderate anemia is a frequent finding. Beriberi is often associated with other diseases.

Diagnosis

Until a simple specific laboratory test for beriberi becomes available, a tentative diagnosis must depend upon the interpretation of a reliable history and a careful physical examination. In obtaining and evaluating the history, the physician should bear in mind that beriberi occurs particularly among the following groups:

1. The indigent and persons who have erroneous dietary habits and idiosyncrasies (20, 23, 24, 26, 27, 38, 39, 55). Analysis of the dietary habits of these persons usually shows that they subsist on a diet abundant in over-milled rice, wheat, or corn. Such a diet is high in carbohydrates and relatively low in proteins, minerals, and vitamins. Oftentimes the total caloric intake is correspondingly low. Such persons rarely eat meat, eggs, milk, fish, or fresh fruits and vegetables in more than meager quantities.

2. Persons who have organic disease (39, 43, 56, 57, 59, 61, 64, 72, 73). Such persons may or may not have had an opportunity to eat sufficient amounts of a well-balanced diet, but the disease may affect their appetites or the assimilation and utilization of the essential food substances, thus predisposing to the development of beriberi. In diseases associated with an increased metabolism, the requirement for Vitamin B_1 is increased. This, in turn, predisposes to the development of beriberi.

3. Persons in whom the vitamin B_1 requirement is distinctly above the average because of growth, pregnancy and lactation, or hard physical exertion (38, 39, 43, 50, 52, 53, 54, 75).

In making a diagnosis of uncomplicated beriberi, all other causes of peripheral neuritis, organic heart disease, and nephritis must be excluded. It is well to keep in mind, however, that beriberi may be present along with other organic diseases.

Adult beriberi. The typical adult case, characterized by an enlarged heart, peripheral neuritis, edema, and tenderness and atrophy of the muscles, is easily diagnosed. The mild case is much more common than the classical one, but the manifestations

vary so greatly that the disease may be recognized only with difficulty. The finding of unexplained peripheral neuritis is indication that the diet of the patient for the past six months should be investigated. If the patient has eaten largely of polished rice or white bread, one must seriously consider the diagnosis of beriberi. When the diagnosis of beriberi is once established, other persons (in the family or institution) receiving the same diet should be examined to determine whether or not they too have incipient beriberi. In making such a survey, one can often detect many mild cases by keeping in mind the following facts noted by Vedder (80): 1. Slight pressure over the muscles of the calf causes pain. 2. Patients with beriberi often have areas of anesthesia over the anterior surface of the tibia. 3. Any modification of the patellar reflexes is suspicious. 4. If a patient with beriberi squats upon his heels after the Oriental manner of sitting, he may experience pain and an inability to arise without using his hands.

The following cardiac symptoms as described by Wenckebach (84) are particularly useful in making a diagnosis of the acute pernicious type of beriberi:

1. Enlargement of the heart by percussion, auscultation and X-ray examination.
2. The presence of murmurs, chiefly systolic but also presystolic with a resonant first sound. The murmurs are disproportionately increased by exercise.
3. Visible and palpable throbbing pulsations over the heart, best felt just to the left of the sternum.
4. Bounding pulse and thrill over the great arteries.
5. Over-distended neck and arm veins and, without exception, a painful, swollen liver. In the most severe cases liver pulsation.

Infantile beriberi. Infants who are restricted to the milk from mothers with beriberi usually develop the disease during the first three months of life. In addition to the cardiac symptoms as given by Wenckebach and described above, the diagnosis of infantile beriberi is made on the basis of the following objective findings: constipation, diminution in the volume of urine, rigidity of the body, weakness, edema, cyanosis, a peculiar whine, irritability, and tachycardia. Response to adequate treatment is rapid.

Prognosis

A favorable course depends upon an early diagnosis followed by intensive and persistent treatment. If untreated or incompletely treated, beriberi or resulting secondary diseases are likely to produce incapacitation or death. The mortality rate varies from 5 to 50 per cent, depending upon the severity

of the beriberi in the group observed and the treatment used. Patients do not die from peripheral neuritis alone, but recovery may be slow. In some instances where the nervous system is extensively damaged, complete regeneration never occurs. The mortality rate is high among patients having acute cardiac involvement (39, 80). The immediate outlook is grave in any patient who develops cardiac symptoms or refuses food or treatment. Once the disease, whether mild or severe, has been remitted, the prognosis is good if the patient continues to ingest, assimilate, and utilize adequate amounts of a well-balanced diet. Recovery is nearly always rapid and complete in infants who are given early and persistent treatment (80).

DISEASES IDENTICAL WITH OR CLOSELY RELATED TO WET BERIBERI

Ship Beriberi

Ship beriberi is rarely seen today and is mentioned here mainly for its historical interest. Vedder (38), in his monograph on "Beriberi," gives a very interesting description of this disease, and it is largely from this work that the following information has been obtained. During the long sea voyages in the era of sailing vessels there appeared among the European and American members of the crew a disease which, because of its similarity to true Oriental beriberi, was named *ship beriberi.* During the last two decades of the nineteenth century, epidemics of ship beriberi were reported frequently, and many investigations were carried out to determine its cause (85, 86, 87, 88, 89, 90, 91).

The symptomatolgy of ship beriberi is similar to that of wet beriberi and consists of loss of appetite; constipation; weakness; edema, particularly of the legs; numbness of the feet; dyspnea; and palpitation of the heart often followed by cardiac failure and death. Since the sailors were usually buried at sea, little information has been obtained from postmortem studies. The relative infrequency of peripheral neuritis led some investigators to believe that ship beriberi was not identical to Oriental beriberi, but today most authorities agree with Vedder that there are no outstanding differences between ship beriberi and true Oriental beriberi of the wet type, provided it is admitted that wet beriberi and dry beriberi are two different forms of the same disease. In etiology as well as in symptomatology, ship beriberi and true beriberi are very similar, if not identical. The sailors lived for months at a time on monotonous diets of white flour, hardtack, and salt meats. The symptoms characteristically became worse, and increased numbers of the crew fell victims so long as the ship remained at sea and a choice of foods was not

available. Within eight to fourteen days after the ship arrived in port and additional foods could be obtained, recovery was complete in most cases. Incomplete recovery rarely occurred, although Vedder reports a few cases in which signs of true neuritis remained. It is generally believed today that ship beriberi, like true beriberi, is caused by a deficiency of vitamin B_1 in the diet (39), although it seems clear that a coexistence of scurvy must have occurred often.

With the passing of the long sailing voyages and with the adoption of facilities for cold storage of food on ships, ship beriberi as a special problem has disappeared. The treatment and prevention of ship beriberi are presumably the same as for true beriberi (see Chapter VI).

Epidemic Dropsy

Epidemic dropsy, a disease closely related to, if not identical with, beriberi, was first described as beriberi in India. In the following year, 1877, MacLeod (92) gave it its present name. This disease is most prevalent in India, but Mann (93), in 1920, described an "oedema disease" in Haiti, which in all probability was the same disease as epidemic dropsy. Since 1908, when Pearse (94) suggested that epidemic dropsy is the same disease as Oriental beriberi, much disagreement has existed over the identity of the two diseases.

The symptomatology of epidemic dropsy differs somewhat from that of true beriberi in that the former is associated with fever, hemorrhages, and glaucoma (38). Fever occurs in 50 per cent of the cases and may be due to the coexistence of malaria or other infections. Hemorrhages occur in about 8 per cent of the cases and may be due to a lack of cevitamic acid. As yet no information as to the cause of the glaucoma is available. The edema and effusions are identical with those of beriberi, and the pathological findings are essentially the same.

Our present standing in regard to the etiology of epidemic dropsy was well summarized by the editor of the Indian Medical Gazette in 1935 as follows: "Many theories regarding the cause of the disease have been formulated, have lived their day and become history, and have been revived again." Recently Lal and Roy (95) revived one of the older theories and concluded that epidemic dropsy, as seen in Bihar, Bengal, Assam, and Orissa, is caused by a deleterious substance which is present in certain consignments of mustard oil. Most authorities, however, now agree that epidemic dropsy results from a deficiency in the diet. Although the disease does occur frequently among the orthodox Hindus who eat no meat or fish, a deficiency of protein is not generally believed to be the underlying cause. Whether

epidemic dropsy, like true beriberi, is caused by a lack of vitamin B_1 can be determined only when large amounts of crystalline vitamin B_1 have been administered to a large series of cases. The principles of both treatment and prevention are the same as those for beriberi (see Chapter VI).

Hunger Edema

According to Prinzing (96), in his history of epidemics resulting from wars, hunger edema was first noted at the time of the Peloponnesian War, from 430 to 425 B.C. Since that time this disease has been reported during many wars and famines and among prisoners of many countries under various names, such as "war edema," "famine edema," "starvation edema," and "prison edema" (92, 93, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108). This disease occurs most frequently among persons performing hard physical labor while being subjected to prolonged underfeeding.

Hunger edema is very similar to and probably closely related to the edema of beriberi (39, 107), cachexia, and pernicious anemia (39). It is most common in the feet and legs and involves the face in about 50 per cent of the cases (39, 107). It may be associated with bradycardia, weakness, asthenia, polyuria, neuritis, muscular weakness, and disturbances of the alimentary tract (39, 107).

The exact cause of the individual symptoms has not been determined, but it is generally believed that they result from prolonged underfeeding of a diet low in protein and calories and high in salt and fluid content, rather than from a diet deficient in a particular vitamin (39, 107). All symptoms of hunger edema respond rapidly to a well-balanced diet. Whether vitamin B_1 plays a causative role in the development of hunger edema is not known. Schittenhelm and Schlecht (109) failed to obtain improvement in cases treated with the vitamin B complex. Bruckman, D'Esopo, and Peters (110) found that the serum proteins were low in patients with malnutrition and evidences of protein starvation due to various causes, and demonstrated further that the albumin fraction alone suffers in the reduction of protein. Now that famines are following in the wake of wars in many parts of the world, controlled studies with crystalline vitamin B_1 should be carried out on a large series of persons with hunger edema, in order to determine the relationship, if any, between a lack of Vitamin B_1 and the development of this form of edema.

Sporadic Cases in Association with Malnutrition

Sporadic cases of beriberi are reported in the literature from time to time (111, 112, 113, 114). However, isolated cases

are often not recognized as true beriberi. The following reports describe typical cases. In 1925 Kepler (55) reported a case in a Negress who restricted herself to a diet of raw starch. At the end of three years she developed polyneuritis with edema and an enlarged heart. One of the authors (115) observed the development of beriberi in a woman who restricted her diet to carbohydrates and fats. Following treatment with large amounts of yeast, the symptoms disappeared. In 1934 Urmy, Ragle, Allen, and Jones (61) demonstrated that beriberi may follow a short-circuit operation on the small intestine and may be cured by disconnecting the intestinal anastomosis. Their cases show that beriberi may result from a lack of proper absorption. Many persons suffering from malnutrition have signs and symptoms clinically indistinguishable from those of beriberi. Now that crystalline vitamin B_1 is available to the physician, it is expected that this substance will be administered to such cases under carefully controlled conditions. In that way alone can we determine whether these sporadic cases are true beriberi.

Prevention and Treatment

The prevention and treatment of all types of beriberi and diseases closely resembling beriberi are discussed in Chapter VI.

BIBLIOGRAPHY

1. Macgowan: A History of China. 1879. Quoted from Vedder, E. B.: Beriberi. William Wood and Company, New York, 1913.
2. Bontius, Jacobus: De medicina Indorum, libri iii, Cap. 1. De Paralyseos Quadam Specie Quam Indigenae Beriberi Vocant. Lugdum, Batavia, 1645.
3. Rogers, C.: De Hydrope Asthmatico in Ceylonia Grassante Beriberia Dicto Complectens. Edinburgi, 1808.
4. Malcolmson, J. G.: A Practical Essay on the History and Treatment of Beriberi. Madras, 1835.
5. Hirota, Z.: Ueber die durch die Milch, der an Kakke Leidenden Frauen, Verursachte Krankheit der Säuglinge. Centralbl. f. innere Med. *19*, 385, 1898.
6. Jansen, B. C. P., and Donath, W. F.: Isolation of Anti-Beriberi Vitamin. Mededeel. Dienst Volksgezondheid Nederland.-Indië *16*, 186, 1927.
7. Williams, R. R., Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin. J. Am. Chem. Soc. *56*, 1187, 1934.
8. Williams, R. R., and Cline, J. K.: Synthesis of Vitamin B_1. J. Am. Chem. Soc. *58*, 1504, 1936.
9. Hermano, A. J., and Eubanas, F.: The Treatment of Human

Beriberi with Crystalline Antineuritic Vitamin. Philip. J. Sci. *57*, 277, 1935.

10. Hashimoto, H.: Acute Pernicious Form of Beriberi and Its Treatment by Intravenous Administration of Vitamin B_1, with Especial Reference to Electrocardiographic Changes. Am. Heart J. *13*, 580, 1937.
11. Murakoshi, Y.: Case of Cardiac Beriberi Cured by Administration of Crystallized Vitamin B. Bull. Nav. M. A., Japan (Abstr. Sect.) *25*, 62, 1936.
12. Matsumura, S., Kakinuma, G., Kawashima, K., Tanikawa, K., Ochiai, S., Miyata, R., Fujisaki, K., Kanao, R., Noguchi, K., Aoki, K., Sato, F., Ito, K., and Suzuki, M.: The Etiology of Beriberi. J.A.M.A. *92*, 1325, 1929.
13. Ogata, T., Kawakita, S., Suzuki, T., Kagoshima, S., and Oka, C.: The Pathogenesis of Beriberi. Japan Med. World, *3*, 106, 1923; abstr. Chem. Abstr. *17*, 2744, 1923.
14. Ogata, T., Kawakita, S., Oka, H., Suzuki, S., and Kagoshima, S.: Kritische Betrachtung über Identität oder Nichtidentität der Vitamin B-Mangelkrankheit mit der Menschlichen Kakke. Deutsch. med. Wochschr. *50*, 527, 1924.
15. Nagayo, Mataro: Beriberi and Rice Neuritis. J.A.M.A. *81*, 1435, 1923.
16. Takaki, K.:
    a. Prevention of Kakke in Japanese Navy. Sei-i-K-wai, August, 1885, and April, 1886.
    b. Special Report on the Kakke Patients in the Imperial Japanese Navy from 1878–86. Sei-i-K-wai *6*, 73, 1887.
17. Eijkman, C.:
    a. Note sur la Prophylaxie du Bériberi. Janus 2, 23, 1897.
    b. Ein Versuch zur Bekämpfung der Beri-Beri. Arch. f. path. Anat. (Virchow's) *149*, 187, 1897.
    c. Polyneuritis gallinarum und Beriberi. Arch. Schiffs-Tropen-Hyg. *15*, 698, 1911.
18. Grijns, G.: Polyneuritis gallinarum. Genneskund. Tijdschr. Nederland.-Indië *41*, 3, 1901.
19. Vorderman, A. G.: Onderzoek naar het verband tusschen den aard der rijstvoeding in de gevangenissen op Iava en Madoera en het voorkomen van beriberi onder de geïnterneeden, Batavia, 1897.
20. Braddon, W. L.: The Cause and Prevention of Beriberi. Rebman Ltd., London, 1907.
21. Fletcher, W.: Rice and Beriberi. J. Trop. Med. and Hyg. *12*, 127, 1909.
22. Ellis, W. G.: Uncured Rice as a Cause of Beriberi. Brit. Med. J. 935, 1909.
23. Fraser, H., and Stanton, H. T.: An Inquiry Concerning the Etiology of Beriberi. Lancet I, 451, 1909.

24. Chamberlain, W. P.
a. Eradication of Beriberi from the Philippine (Native) Scouts by Means of a Simple Change in Their Dietary. Philip. J. Sci., Sect. B, *6*, 133, 1911.
b. Prevention of Beriberi among Philippine Scouts by Means of Modifications in the Diet. J.A.M.A. *64*, 1215, 1915.
25. Thomson, D., and Simpson, G. C.: Treatment of Beriberi. Ann. Trop. Med. and Parasitol. *6*, 53, 1912.
26. Chamberlain, W. P., and Vedder, E. B.:
a. A Contribution to the Etiology of Beriberi. Philip. J. Sci., Sect. B, *6*, 251, 1911.
b. A Second Contribution to the Etiology of Beriberi. Philip. J. Sci., Sect. B, *6*, 395, 1911.
c. The Cure of Infantile Beriberi by the Administration to the Infant of an Extract of Rice Polishings, and the Bearing Thereof on the Etiology of Beriberi. Bull. Manila Med. Soc. *4*, 26, 1912.
27. Chamberlain, W. P., Vedder, E. B., and Williams, R. R.: A Third Contribution to the Etiology of Beriberi. Philip. J. Sci., Sect. B, *7*, 39, 1912.
28. Willcox, W. H.:
a. Beriberi—with Special Reference to Prophylaxis and Treatment. J. Roy. Army Med. Corps *27*, 191, 1916.
b. Rations in Relation to Disease in Mesopotamia. Lancet II, 677, 1917.
c. The Treatment and Management of Diseases Due to Deficiency of Diet:—Scurvy and Beriberi. Brit. Med. J. I, 73, 1920.
29. Hehir, P.: Beriberi. Indian J. Med. Research Spec. Indian Sci. Cong., No. 44–59, 1919.
30. Strong, R. P., and Crowell, B. C.: The Etiology of Beriberi. Philip. J. Sci., Sect. B, *7*, 271, 1912.
31. Caspari, W., and Moszkowski, M.: Beriberifrage. Deutsch. med. Wochschr. *39*, 1479, 1529, 1913.
32. Fraga, C.: Beriberi. Brazil Med. *33*, 49, 1919.
33. Riddell, J. D., Smith, C. H., and Igaravidez, P. G.: Beriberi at U.S. Army Base Hospital, San Juan, Porto Rico: Laboratory Investigations and Clinical Manifestations of Sixty Cases. J.A.M.A. *72*, 569, 1919.
34. Hehir, P.: Beriberi. Proc. Asiat. Soc. Bengal *15*, 212, 1919; Mesopot. Comm. Rep. Appendix III, 1917.
35. Ohomori, K.: Studies on the Cause and Treatment of Beriberi in Japan. Japan Med. World *3*, 231, 1923.
36. Jolliffe, N., Colbert, C. N., and Joffe, P. M.: Observations on the Etiologic Relationship of Vitamin B (B_1) to Polyneuritis in the Alcohol Addict. Am. J. Med. Sc. *191*, 515, 1936.
37. Kagawa, S., and Naito, H.: Die Experimentelle B_1-Avitaminose

beim Menschen. Jap. J. M. Sc., VIII, Int. Med., Pediat. & Psychiat. *4*, 128, 1936.

38. Vedder, E. B.: Beriberi. William Wood and Company, New York, 1913.
39. Stitt, E. R.: The Diagnostics and Treatment of Tropical Diseases, 5th ed. P. Blakiston's Son and Company, Philadelphia, 1929.
40. Sturm, A.: Nachtrag zur Umfrage über die Behandlung der Basedowschen Krankheit. Med. Klin. *32*, 59, 1936.
41. Means, J. H., Hertz, S., and Lerman, J.: Nutritional Factors in Graves' Disease. Ann. Internal Med. *11*, 429, 1937.
42. Funk, C.: The Vitamins, Translated by H. E. Dubin. Williams and Wilkins Company, Baltimore, 1922.
43. Haden, Russell L.: Multiple Specific Nutritional Deficiency Disease in the Adult. J.A.M.A. *106*, 261, 1936.
44. Bentley, A. J. M.: Beriberi, Its Etiology, Symptoms, Treatment and Pathology. Y. J. Pentland, Edinburgh, 1893.
45. Yacoub, K.: Notes on Three Cases of Beriberi Following Relapsing Fever and Due to Prolonged Feeding on Condensed Milk. Practitioner *100*, 290, 1918.
46. Yang, C. S., and Huang, K. K.: Beriberi in Nanking. Chinese Med. J. *48*, 20, 1934.
47. Platt, B. S., and Gin, S. Y.: Some Observations on a Preliminary Study of Beriberi in Shanghai. Trans. IXth Congr., Far East. Asso. Trop. Med. *2*, 407, 1934.
48. Cannon, A.: Some Observations on Beriberi. Brit. M. J., 2, 852, 1929.
49. Walshe, F. M. R.: On the Deficiency Theory of the Origin of Beriberi in the Light of Clinical and Experimental Observations on the Disease, with an Account of a Series of Forty Cases. Quart. Jour. Med. *11*, 320, 1918.
50. Strauss, Maurice B.: Nerve Disorders Arising from Defective Nutrition. New England J. Med. *215*, 1164, 1936.
51. Elsom, K. O., and Sample, A. B.: Macrocytic Anemia in Pregnant Women with Vitamin B Deficieney. J. Clin. Invest. *16*, 463, 1937.
52. Sure, B.: Dietary Requirements for Fertility and Lactation; Does Amount of Fat in Diet Influence Vitamin B Requirements for Lactation? Proc. Soc. Exper. Biol. & Med. *30*, 622, 1933.
53. Macy, I. C., Outhouse, J., Graham, A., and Long, M. L.: Human Milk Studies: III. Quantitative Estimation of Vitamin B. J. Biol. Chem. *73*, 189, 1927.
54. Tarr, E. M., and McNiele, O.: Relation of Vitamin B. Deficieney to Metabolic Disturbances During Pregnancy and Lactation. Am. J. Obst. & Gynec. *29*, 811, 1935.
55. Kepler, E. J.: Beriberi from a Diet of Raw Starch. J.A.M.A. *85*, 409, 1925.

56. Haas, S. V.: Beriberi in Late Infancy: The Result of Celiac Disease: Arch. Pediat. *46*, 467, 1929.
57. Keefer, C. S., and Yang, C. S.: The Treatment for Secondary Anemia; A Study of the Results in 126 Cases. Arch. Int. Med. *48*, 537, 1931.
58. Eusterman, G. B., and O'Leary, P. A.: Pellagra Secondary to Benign and Carcinomatous Lesions and Dysfunction of the Gastro-intestinal Tract: Report of Thirteen Cases. Arch. Int. Med. *47*, 633, 1931.
59. Jones, C. M.: Peripheral Complications of Ulcerative Colitis. M. Clin. North America *16*, 919, 1933.
60. Kohn, S.: Mitteilungen über Einige Seltenere Krankheitsfalle aus der Privaten Praxis. Präger med. Wochschr. *35*, 584, 1910.
61. Urmy, T. V., Ragle, B. H., Allen, A. W., and Jones, C. M.: Beriberi Secondary to Short-Circuited Small Intestine. New England J. Med. *210*, 251, 1934.
62. Poljakoff, W., and Choroschko, W.: Polyneuritis und Bacterium Coli. Deutsche med. Wochschr. *33*, 1452, 1907.
63. McVicar, C. S.: Jaundice Associated with Peripheral Neuritis. M. Clin. North America *11*, 1415, 1928.
64. Strauss, M. B.: The Role of the Gastro-intestinal Tract in Conditioning Deficiency Disease. J.A.M.A. *103*, 1, 1934.
65. Mackie, T. T., and Pound, R. E.: Changes in the Gastro-intestinal Tract in Deficiency States, with Special Reference to Small Intestine; Roentgenologic and Clinical Study of 40 Cases. J.A.M.A. *104*, 613, 1935.
66. Mackie, T. T.: Ulcerative Colitis; the Factor of Deficiency States. J.A.M.A. *104*, 175, 1935.
67. Weller, G. L., Jr.: Clinical Aspects of Vitamin B-Complex Deficiency in Association with Diseases of Gallbladder; Report of Instance. Am. J. Digest. Dis. & Nutrition *3*, 324, 1936.
68. Libert, E.: Sténose Ulcéreuse du Pylore Accompagnée de Pseudotabes Polynévritique chez un Syphilitique Ancien. Arch. d. mal. de l'app. digestif *20*, 493, 1930.
69. Klippel, M., and Weil, M. P.: Les Complications Nerveuses de l'Ulcère de l'Estomac; Polynévrites et Pseudotabes Polynévritiques. Press méd. *19*, 753, 1911.
70. Couréménos and Conos, B.: Polynévrite dans l'Ulcère Gastrique. Encéphale *2*, 423, 1909.
71. Duvernay: Névrites Périphériques Toxiques Liées à de la Rétention Gastrique. Lyon méd. *109*, 53, 1907.
72. Hartsock, C. L.: The Role of Deficiency Disease in Diseases of the Gastro-intestinal Tract. Rev. Gastro. *3*, 111, 1936.
73. Muller, H.: Relative Lack of Habitual Alimentation and of Vitamins in Diets, Particularly Vitamin B_1; Need for Addition of Vitamin B_1 to Diet, Especially in Gastro-intestinal Diseases. Nutrition *5*, 425, 1935.

74. Schioedt, E.: Gastro-intestinal Affections from Vitamin Deficiency. Acta Med. Scandinav. *84*, 456, 1935.
75. Cowgill, G. R.: The Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
76. Guerrero, M. S.: Etiología del Beriberi en Los Niños de Pecho. Bull. Manila M. Soc. 2, 243, 1910.
77. Andrews, V. L.: Infantile Beriberi. Philip. J. Sci., Sect. B, *7*, 67, 1912.
78. Waring, J. I.: Beriberi in Infants. Am. J. Diseases Children *38*, 52, 1929.
79. Albert, J.: Studies on Infantile Beriberi Based on Five Hundred Fourteen Cases. Philip. J. Sci. *45*, 297, 1931.
80. Vedder, E. B.: Beriberi and Epidemic Dropsy. Tice's Practice of Medicine, IV. W. F. Prior Company, Hagerstown, Maryland, 1936.
81. Platt, B. S., and Lu, G. D.: Chemical and Clinical Findings in Beriberi with Special Reference to Vitamin B_1 Deficiency. Quart. J. Med. *5*, 355, 1936.
82. Weiss, S., and Wilkins, R. W.: The Nature of the Cardiovascular Disturbances in Nutritional Deficiency States (Beriberi). Ann. Int. Med. *11*, 104, 1937.
83. Goodhart, R., and Jolliffe, N.: Cardiovascular Disturbances in the Alcohol Addict: The Role of Nutritional Deficiencies in Their Production. (In press.)
84. Wenckebach, K. F.: Heart and Circulation in a Tropical Avitaminosis (Beriberi). Lancet, II, 265, 1928.
85. Shattuck, F. C.: A Curious Epidemic on Board the Nelly Swift. Boston Med. and Surg. J. *105*, 400, 577, 1881.
86. Roosevelt, J. W.: A Short Study of Beriberi, with a Report of Cases Observed in Bellevue Hospital. Med. Record, N. Y., *31*, 210, 1887.
87. Putnam, J. J.: A Form of Polyneuritis, Probably Analogous to or Identical with Beriberi, Occurring in Sea-faring Men in Northern Latitudes. J. Nerv. and Ment. Dis., N. Y., *17*, 495, 1890.
88. Birge, W. S.: Cases of Beriberi Occurring in the Provincetown Grand Bank Fishing Fleet. Boston Med. and Surg. J. *123*, 464, 1890.
89. Bullmore, C.: Beriberi. Ship Cases at Falmouth. Lancet, II, 873, 1900.
90. Report of the Norwegian Beriberi Investigation Commission. Skibe, Kristiania, 1902. Quoted from Vedder (80).
91. Nocht: Ueber Segelschiff Beriberi. Festschrift zum 60 Geburtstag von Robert Koch. Jena, p. 203, 1903.
92. MacLeod, K.: Epidemic Dropsy in Calcutta. Indian M. Gaz. *16*, 148, 1881.
93. Mann, W. L., Helm, J. B., and Brown, C. J.: An Edema Disease in Haiti. J.A M A. *75*, 1416, 192

94. Pearse, F.: On the Identity of Beriberi and Epidemic Dropsy. Indian M. Gaz. *43*, 128, 1908. Also J. Trop. M. and Hyg. *11*, 69, 1908.
95. Lal, R. B., and Roy, S. C.: Epidemiology of Epidemic Dropsy. Brit. M. J. *1*, 1110, 1937.
96. Prinzing, F.: Epidemics Resulting from Wars; Edited by Harold Westergaard. H. Milford, New York, 1916.
97. Maliwa, E.: Bemerkungen zur "Oedemkrankheit." Wien. klin. Wochschr. *30*, 1477, 1917.
98. Wheeler, W. A.: The Epidemic of Beri-Beri in the Boer Camp at St. Helena. Brit. Med. J. *2*, 1258, 1902.
99. Falta, W.: Ueber das Kriegsödem. Wien. klin. Wochschr. *30*, 1637, 1917.
100. Patterson, A. B.: Starvation Edema. Med. Rec., Nov., 715, 1899.
101. Mayer, M. B.: Nutritional Edema and War Dropsy. J.A.M.A. *74*, 934, 1920.
102. Rumpel, T.: Zur Aetiologie der Oedemkrankheiten in Russischen Gefangenenlagern. Münch. med. Wochschr. *62*, 1021, 1915.
103. Knack, A. V.: Beitrag zur Klinik der sogenannten Oedemkrankheit. Wien. klin. Wochschr. *29*, 1015, 1916.
104. Vandervelde, P., and Cantineau, G.: La Déportation des Civils Flamands en 1916. Considérations Médicales. Première Série de Deux Cent Cas Soignés à l'Hôpital Saint-Pierre. Bull. Acad. roy. de méd. de Belg., Brux., *29*, 129, 1919.
105. Park, F. S.: War Edema (Kriegsoedem). J.A.M.A. *70*, 1826, 1918.
106. Enright, J. I.: War Edema in Turkish Prisoners of War. Lancet I, 314, 1920.
107. Aykroyd, W. R.: Famine Oedema. Brit. M. J. *2*, 247, 1930.
108. Parker, H. B.: A Report on Beriberi in the County Jail at Elizabeth. N. J., U. S. Health Repts. *29*, 339, 1914.
109. Schittenhelm, A., and Schlecht, H.: Die Oedemkrankheit. Springer, 1919, Z. exp. Med. *9*, 1, 1919.
110. Bruckman, F. S., D'Esopo, L. M., and Peters, J. P.: The Plasma Proteins in Relation to Blood Hydration. IV. Malnutrition and the Serum Proteins. J. Clin. Invest. *8*, 577, 1930.
111. Little, J. M.: Beriberi. J.A.M.A. *63*, 1287, 1914.
112. Holmes, B., and Retinger, J.: An Apparent Recovery of a Chicago Chinaman from Beriberi after Three Doses of Vitamin-Containing Extract of Rice Polishings. Illinois Med. J. *29*, 178, 1916.
113. Reed, A. C.: Beriberi: Report of Cases. J.A.M.A. *68*, 116, 1917.
114. Abdou, N. T.: Descriptive Picture of Beriberi. J.A.M.A. *71*, 1298, 1918.
115. Spies, T. D.: Unpublished observations.

CHAPTER III

VITAMIN B_1 AND THE POLYNEURITIS OF CHRONIC ALCOHOLISM, PREGNANCY, KORSAKOFF'S SYNDROME, PELLAGRA, SPRUE, PERNICIOUS ANEMIA, COLITIS, DIABETES, TUBERCULOSIS, PORPHYRINURIA, SENILITY, MALIGNANCY, AND CIRRHOSIS

Polyneuritis in the Chronic Alcoholic Addict

Chronic alcoholic addicts frequently develop polyneuritis which clinically and pathologically is indistinguishable from the polyneuritis of beriberi described in the previous chapter. "Alcoholic" polyneuritis was first described about one hundred and fifty years ago by the English physician, John Coakley Lettsom (1). He, as well as James Jackson (2), author of the second classic description of this condition, attributed it to a direct neurotoxic effect of the alcohol. There was full acceptance of this hypothesis until 1928, when Shattuck (3) suggested that the polyneuritis occurring in the alcoholic addict might result from a deficiency in the diet, a theory which is accepted by the majority of clinicians today. Shattuck stated that "alcoholic polyneuritis, as well as the polyneuritis frequently associated with other debilitating diseases which often interfere with nutrition, such as diabetes, cancer, tuberculosis, syphilis, pregnancy, and the marasmus of infants, might be caused by a deficiency of vitamin B in the diet or by failure to assimilate that vitamin if it were present in adequate amounts in the diet." He further stated that the polyneuritis found in association with these debilitating diseases "might be properly regarded as true beriberi and not merely as complications or sequelae of other diseases." Minot, in 1929 (4) and again in 1931 (5), suggested that many cases of polyneuritis are probably dependent, in part at least, on an inadequate diet, and stressed the value of high vitamin diets in the treatment of such cases. Subsequent clinical studies served to strengthen the belief that "alcoholic" polyneuritis is the same as the polyneuritis of beriberi and develops in a similar manner; that is, from a deficiency of the antineuritic factor, vitamin B_1. Their absolute identity, however, awaits confirmation. Time and space prohibit a complete review of the contributions which have been made toward increasing our knowl-

edge of the etiology and treatment of "alcoholic" polyneuritis, but the most outstanding studies of the past few years will be considered briefly.

Evidence of a relationship between lack of vitamin B complex and the development of "alcoholic" polyneuritis. In 1932 Meyer (6), from a careful study of the dietary habits of several cases of "alcoholic" polyneuritis, concluded that as a rule the alcoholic addict who develops polyneuritis has an inadequate intake of food and vitamins. He, like Shattuck, felt that the essential protective substance might be vitamin B and noted that the clinical manifestations and pathologic findings were very similar to those found in beriberi.

In the following year Wechsler (7) observed improvement in three chronic alcoholic addicts with polyneuritis, following the administration of foods rich in vitamins. Although he was not able to express any final opinion concerning the nature of the food deficiencies in the cases reported, he suggested that a lack of vitamin B_1 or B_2 (G) was probably the underlying cause of the polyneuritis. In conclusion he stated that many cases of polyneuritis which have hitherto been regarded as due solely to a specific cause such as alcohol, arsenic, lead, and phosphorus may have underlying it another and possibly determining factor in avitaminosis. He called attention to the fact that the general pathologic changes, which are degenerative rather than inflammatory, are similar to the degenerative changes in avitaminosis.

In 1933 Minot, Strauss, and Cobb (8) suggested that the deficiency which is responsible for the development of polyneuritis "can be attributed not only to inadequate intake [of food] but also to the state of the gastro-intestinal tract and the presence of factors that can inhibit the effectiveness of nutritional elements." They arrived at this conclusion after studying the dietary histories of and performing gastric analysis on a group of patients with "alcoholic" polyneuritis. These investigators found that the diet, in many instances, was deficient in calories as well as in protein, minerals, and vitamins. Fractional gastric analyses showed a 33 per cent incidence of gastric hypochlorhydria and a 50 per cent incidence of gastric achlorhydria, in contrast to an expected hypochlorhydria of only 12 per cent in a group of normal individuals of the same age group and sex ratio. In view of the frequent association of hypochlorhydria with certain deficient states such as pernicious anemia, pellagra, and sprue, in which it is encountered in 99 per cent, 64 per cent, and 50 per cent respectively (9), the incidence of achlorhydria in the alcoholic addict with peripheral neuritis seemed particularly significant. By treatment with a diet rich in protein, minerals, and vitamins, and supplemented with cod liver oil

and autolyzed yeast or extractives, recovery was "observed more uniformly and with greater rapidity than previously." They stated that the belief that dietary deficiency, especially the lack of vitamin B_1, plays an important role in the production of "alcoholic" polyneuritis is favored by the grossly deficient diets taken by the patients for a long period of time, the similarity of the lesions and symptoms to those of beriberi, and the more satisfactory improvement with a diet rich in proteins and vitamins than with any other form of therapy.

Meanwhile Spies and DeWolf (10), while studying the relationship between severe alcoholism and the development of "alcoholic" pellagra, had noted that the clinical manifestations of polyneuritis did not become worse in so-called "alcoholic" pellagrins taking large quantities of whisky if they ate large amounts of a well-balanced diet. Strauss (11), using the same approach, conducted controlled studies on the effect of alcohol on patients with "alcoholic" polyneuritis unassociated with pellagra. Ten such patients were allowed to continue their customary intake of hard liquor, from 1 pint to 1 quart daily, on condition that they consume daily a high caloric, high vitamin diet and 18 grams of Vegex, or 30 Harris yeast-vitamin tablets, or 90 grams of dried brewers' yeast. In addition, each patient received 10 cc. of intramuscular vitamin B concentrate (Lilly) each day, and 10 cc. of intramuscular liver extract solution (Lilly N.N.R.) each week. Under this regime the polyneuritis improved in every instance. The author concluded that "'alcoholic' polyneuritis does not result primarily from a direct neurotoxic effect of alcohol, but is probably the result of a dietary deficiency, possibly conditioned in some cases by disturbed gastro-intestinal function." It is suggested that "alcoholic" polyneuritis be regarded as similar to the polyneuritis of beriberi and treated accordingly.

At about the same time, Blankenhorn and Spies (12) reported the results of curative and preventive studies on fifty cases of "alcoholic" polyneuritis, forty-six with pellagra and four with chronic alcoholism unassociated with pellagra. Every patient gave a history of an inadequate diet over a long period of time. Following treatment with a high caloric, high vitamin diet, often supplemented with either dry powdered brewers' yeast (Harris) or liver extract (Parke, Davis & Company), all fifty patients eventually became free from symptoms. Studies were continued on some of the fifty patients after discharge from the hospital. Fifteen were induced to stop drinking and to eat three full meals each day. When these people were last observed, more than a year after discharge from the hospital, they had had no recurrence of the pellagra or the neuritis. Twenty other pellagrins who had had repeated attacks of peripheral

neuritis were allowed to live at home and drink as much alcohol as they pleased. Five of the twenty were allowed to eat as they wished and were given frequent large injections of liver extract. The remaining fifteen received an adequate diet often supplemented with dry powdered brewers' yeast. None of these twenty patients developed any evidence of pellagra or peripheral neuritis during the subsequent year of observation. In summary, the authors stated that: 1. The peripheral neuritis associated with "alcoholic" pellagra or with chronic alcoholism without pellagra may improve in spite of the patients' drinking large quantities of whisky, provided an adequate diet is also ingested. 2. Persons who are prone to relapses of the peripheral neuritis have no return of symptoms, provided they do not drink or, if they do drink, provided they eat adequately or take large amounts of either yeast or liver extract.

Thus far, the results of investigative work strengthened the hypothesis that "alcoholic" polyneuritis results from inadequate ingestion or assimilation of some essential substance or substances and failed to give any indication that alcohol has a direct harmful effect on the nerves. Furthermore, it seemed likely that vitamin B_1, or at least some part of the vitamin B complex, was the factor involved. The next step was added by Cowgill (13), who emphasized a quantitative relationship between the number of calories ingested and the amount of vitamin B_1 required, in the diet of animals. Using Cowgill's formula, Jolliffe, Colbert, and Joffe (14) estimated quantitatively the vitamin B_1 and caloric intake of forty-two alcoholic addicts, twenty-six of whom had polyneuritis. They found that if the caloric value of the ingested alcohol was included in the estimation of the vitamin-calorie ratio, a definite correlation existed between the inadequacy or adequacy of the vitamin B_1 intake and the presence or absence of polyneuritis. That is, that every alcoholic addict with polyneuritis had an estimated inadequate vitamin B_1 intake, whereas no alcoholic addict with an estimated adequate vitamin B_1 intake had polyneuritis. The authors concluded that alcohol has no direct toxic action (chronic) on the peripheral nerves, and that a deficiency of vitamin B_1 is the cause of polyneuritis in the alcoholic addict.

Subsequently, Jolliffe and Colbert (15) gave to twenty-eight alcoholic addicts with uncomplicated polyneuritis, diets containing slightly inadequate or barely adequate quantities of vitamin B_1 or diets containing either approximately two times or four times the estimated vitamin B_1 requirement. They found that those who received borderline amounts of vitamin B_1 showed no improvement in the objective signs of peripheral neuritis over a period of a month. Those who received approximately twice their predicted requirement of vitamin B_1 showed

improvement which was, however, not as rapid or as dramatic as in those patients who received four times their vitamin B_1 requirement. Since Vegex, a complex preparation of autolyzed brewers' yeast containing vitamin B_1 as well as vitamin B_2, was used to increase the vitamin B intake, the authors suggest that there may be a fraction in the vitamin B complex, other than vitamin B_1 itself, which augments the action of vitamin B_1 on the polyneuritis in the alcoholic addict. More recently, Jolliffe and Goodhart (16) have pointed out that cardiovascular disturbances are seen in approximately one third of the chronic alcoholic addicts who show vitamin B_1 deficiency in the form of peripheral neuritis, and concluded that these cardiovascular disturbances are probably manifestations of vitamin B_1 deficiency.

Recently Romano (17) reported the results of a clinical study of the deficiency syndromes associated with chronic alcoholism. Of the 77 patients (58 per cent of the total number) who showed evidence of some form of neuritis, 61 (79 per cent) gave a history of an inadequate diet. Every patient with mild or severe involvement of the peripheral nerves was treated with a high caloric (4000 to 5000 calories daily), high vitamin diet supplemented with powdered brewers' yeast tablets (4 gm. daily), wheat germ preparations (30 to 60 gm. daily), and either vitamin B_1 or liver preparations given parenterally in daily doses. Of the 77 patients with neuritis, 5 (6.4 per cent) failed to respond to such therapy, 47 (61 per cent) showed partial improvement, and 25 (32.4 per cent) showed relief of all symptoms during their period of hospitalization. The author concluded that vitamins B_1 and B_2 (G) are of definite value in the treatment of the deficiency syndromes associated with chronic alcoholic addiction.

Studies with crystalline vitamin B_1. The identification and synthesis of vitamin B_1 by Williams and his associates (18, 19), making possible the manufacture of large quantities of crystalline vitamin B_1, have led to rapid progress in clinical research on this subject. In 1935 Blankenhorn and Spies (12) reported improvement in the peripheral neuritis of a case of "alcoholic" pellagra following the administration of vitamin B_1 crystals (Merck). The patient was given a pellagra-producing diet *ad libitum,* as previous experience had shown that the neuritic symptoms of pellagra often progress on such a diet. In addition, he was given 3 ounces of whisky before each meal, an amount comparable to what he had been drinking before admission to the hospital. The hyperesthesia, excruciating burning of the feet, and shooting pains were variable in their manifestation, but, in general, they progressed. By the end of two months he would not put on his shoes, required a cradle on his bed to keep the sheets away from his feet, and codeine had to be administered each night.

Continuing on the same diet and the same amount of whisky, the patient was given 15 milligrams of vitamin B_1 crystals by mouth daily for the next fifteen days. By the end of the second week, the symptoms had begun to improve slowly but definitely. By the end of the fourth week after the initial administration of vitamin B_1, the patient was able to walk with tightly laced shoes and no longer required a cradle or codeine.

Vorhaus, Williams, and Waterman (20) have also reported favorable results in cases of various types of polyneuritis, including "alcoholic," following the daily oral administration of 10 milligrams of pure crystalline vitamin B_1.

Jolliffe and Colbert (15) observed a rapid response in two alcoholic addicts with polyneuritis following intravenous injections of vitamin B_1. One patient, who had absent ankle-jerks, a bilateral foot-drop, and severe skin hyperesthesia in the lower extremities, showed no improvement when maintained for seventy-four days on a diet which was of borderline adequacy. She was then given 10 milligrams of crystalline vitamin B_1 by intravenous injection each day for five consecutive days. After 20 milligrams had been given, the knee-jerks returned, the foot-drop disappeared, and the sensory status was improved. By the end of the sixth day the patient was able to get out of bed for the first time in eighty days. On the following day her diet was changed to a vitamin-rich one supplemented with 18 grams of Vegex. The crystalline vitamin B_1 (thiamin) was discontinued. Within a week all previously noted symptoms had returned, though they were less severe than formerly. Continuing on the same diet and supplement, she was given 10 milligrams of crystalline thiamin intravenously for each of the next two days. Within twenty-four hours dramatic improvement again took place. There had been no return of symptoms up to the time of the paper's publication eight months later. The second patient, who also received the basal diet of borderline adequacy and, in addition, 18 grams of autoclaved Vegex for a period of twenty-seven days, showed progression of sensory abnormalities. Beginning on the twenty-eighth day, she was given 10 milligrams of crystalline vitamin B_1 intravenously daily for fourteen consecutive days. On the third day, sensory improvement was noted, and on the fourteenth day the knee-jerks had returned. At that time she was placed on a vitamin-rich diet, and up to the time of publication, twenty days later, there had been no recurrence of symptoms.

More recently, Sciclounoff and Broccard (21) reported rapid amelioration of symptoms varying from simple neuritic pain with paresthesia to classical Korsakoff's syndrome, by treatment with vitamin B_1 (Hoffman-La Roche) administered subcutaneously. Improvement in single cases of "alcoholic" poly-

neuritis following the use of vitamin B_1 has been reported by other workers (Neumann (22); Russell (23); Desrochers and Larue (24)). From a recent study, Goodhart and Jolliffe (25) concluded not only that a deficiency of vitamin B_1 is the primary cause of polyneuritis in the alcoholic addict, but also that improvement in the objective signs in such cases "varies directly with the vitamin B intake up to a point of optimum dosage which, though not as yet determined, is definitely more than four times the predicted maintenance requirement." They placed seventeen alcoholic addicts with uncomplicated mild polyneuritis on diets containing four times the predicted vitamin B requirement of a subject weighing 60 Kg. Alternate subjects of this group were then given 10 milligrams of either natural or synthetic crystalline vitamin B_1 each day for ten days. Without exception, all patients receiving the crystalline vitamin B_1 showed more rapid and complete improvement than did those of the control group. No difference in response to the administration of synthetic and natural crystalline thiamin was noted.

It is certain, from the studies thus far reported, that "alcoholic" polyneuritis is at least partially nutritional in origin and that rapid improvement can be brought about by treatment with high vitamin diets, yeast, liver extract and vitamin B_1, administered orally or parenterally. Since, until recently, recovery has followed the administration of substances which contain more than one "accessory" factor, we cannot say conclusively that vitamin B_1 is the sole factor involved. In those instances where pure vitamin B_1 has been given, little attempt has been made to make certain that other factors which were present in the diets administered the patients did not play some part in effecting the cure. Until this is done, we cannot say with definite assurance that a lack of vitamin B_1 is the sole cause of "alcoholic" polyneuritis.

Diagnosis. Persons who regularly drink large quantities of alcoholic beverages and who either actually do not eat a good diet regularly or if they do eat such a diet, do not utilize it because of vomiting, diarrhea, or other gastro-intestinal disturbances, are especially prone to develop polyneuritis. For this reason it is essential that the physician make every effort to obtain a complete and accurate history of the patient's dietary and alcoholic habits. In the experience of the authors it is very difficult to obtain an accurate history from most addicts, as they usually lie or boast. The physician should ascertain not only what food the patient has an opportunity to eat, but also whether he actually does eat and retain it. The symptoms and signs of "alcoholic" polyneuritis are indistinguishable from those of the neuritis of beriberi (see Chapter II).

Treatment and prevention. See Chapter VI for the prevention and treatment of "alcoholic" polyneuritis.

Polyneuritis of Pregnancy

The occurrence of polyneuritis in pregnant women has been mentioned in medical literature since the middle of the nineteenth century, but only recently, through our increased knowledge of the underlying etiological factors, has treatment of this condition been attended with prompt and satisfactory results. General reviews of the polyneuritis of pregnancy have been written recently by Berkwitz and Lufkin (26) and by Plass and Mengert (27), to whose writings the reader is referred for an account of the earlier literature on this subject and details of the symptomatology and pathology of this condition.

Evidence of a relationship between malnutrition and the development of polyneuritis in pregnant women. In 1889, at a time when the majority of writers favored the theory that the polyneuritis of pregnancy results from the presence of a toxic substance, Whitfield (28) reported the first case in English literature and attributed its development to prolonged and excessive vomiting. Extremely interesting, in view of our present concept of the etiology of the polyneuritis of pregnancy, is the fact that Tuilant (29), nearly fifty years ago, suggested that a lack of nourishment, resulting from severe vomiting, was responsible for the development of this neuropathy. Subsequent writers (30, 31, 32, 33, 34, 35) also noted the frequent association of persistent vomiting and the polyneuritis of pregnancy. It was generally believed, however, that the vomiting was indicative of a toxic substance which caused the polyneuritis as well as the vomiting, rather than that the vomiting was the predisposing factor responsible for the development of the polyneuritis. In 1924 Hofmann (36) stated that the polyneuritis occurring in Chinese women during pregnancy and the puerperium was not a new disease but true beriberi. He noted that beriberi developed more frequently in women during the childbearing period than at any other period, and that childbearing seemed to make beriberi a much more serious disease.

In 1930 Whitfield's belief that malnutrition is responsible for the development of the polyneuritis of pregnancy was expressed anew by Wechsler (37). He discussed eight cases of polyneuritis, including one associated with pregnancy. All eight patients suffered from a food deficiency due either to voluntary dietary restriction or to some gastro-intestinal disorder. His conclusion that food deficiency, possibly a deficiency of vitamins, was the probable cause of the polyneuritis of pregnancy as well as of other types of polyneuritis, was strengthened by the fact that

some of the patients recovered when put on diets rich in vitamins. In the same year Theobald (38) stated that it was "possible that the severe neuritis which is sometimes associated with pregnancy in this country [England] may be a form of beriberi." Later, this author recommended that all women, during the latter half of pregnancy, should be assured of an adequate supply of vitamins A, B, C, and D.

In 1933 Strauss and McDonald (39) reported successful treatment of the neuritis of pregnancy by the administration of diets rich in vitamin B. They suggested that since "certain cases of macrocytic (pernicious) anemia of pregnancy lack not merely a dietary factor associated with vitamin B_2, but also an unidentified factor like the one absent in Addisonian pernicious anemia and which is present in normal gastric juice, it is possible that a similar dual mechanism may be at work in certain cases of polyneuritis of pregnancy." Because of this possibility, they recommended the use of liver or liver extracts, both orally and parenterally, in cases not responding to preparations of vitamins B_1 and B_2. The authors stated that all pregnant women with severe vomiting should be given vitamin B as a safeguard against the development of polyneuritis. Strauss and McDonald based their belief that the neuritis of pregnancy is a deficiency disease on the following facts and observations: "First, and of most importance, is the fact that severe vomiting almost always occurs recurrently for many weeks before the onset of polyneuritis. This, of course, prevents the patient from ingesting and absorbing the proper food. Secondly, it has been shown that abnormalities of the gastro-intestinal tract can play an important role in the causation of deficiency diseases, and in pregnancy there is ordinarily a marked decrease of gastric secretory function. Thirdly, in pregnancy the fetus takes all sorts of materials from the maternal organism, no matter what depletion may result to the mother. Fourthly, the clinical and pathologic examination in polyneuritis of pregnancy is identical with that of beriberi and 'alcoholic' polyneuritis and is unlike the more strictly motor nerve involvement due to lead, and triorthocresyl phosphate (jamaica ginger) polyneuritis."

At about the same time Luikart (40) noted the close relationship between the clinical symptoms and pathologic findings of the polyneuritis of pregnancy and those of Korsakoff's syndrome and pointed out that the neuritis associated with Korsakoff's syndrome is supposedly due to the constant vomiting of alcoholism, and ultimately to avitaminosis. Postmortem studies on one case of polyneuritis of pregnancy showed great similarity between pathologic sections of the neurones in the polyneuritis of the vomiting of pregnancy and those in beriberi, pellagra, and scurvy. Before death occurred, the patient reported by

Luikart showed definite remission of many of her symptoms following forced feeding of vitamin B complex for twelve hours.

In the following year Fouts, Gustafson, and Zerfas (41) also demonstrated the therapeutic value of the vitamin B complex in the polyneuritis of pregnancy and advised the parenteral administration of vitamin B to all cases of persistent vomiting of pregnancy. They concluded that the polyneuritis is not the result of a toxemia but is due to a deficiency in the diet. They secured the most outstanding improvement of neurologic symptoms by the administration of large doses of vitamin B_1 in the form of concentrated extract of rice polishings (tiki-tiki extract).

These findings were confirmed in 1936 by Theobald (42), who successfully treated five cases by means of diet. In four of them the cure was brought about solely by the administration of vitamin B_1 concentrate. He concluded that the neuritis resulted from a deficiency of vitamin B_1 in the diet.

As the result of studies on four cases, Bingham (43) in 1936 concluded that "while it [the polyneuritis of pregnancy] is due to a dietary deficiency, especially a lack of vitamin B, it may also be caused by an autointoxication of pregnancy." He advised the use of vitamin B as a preventive of polyneuritis in all cases of pregnancy with much vomiting or malnutrition. Ford (44) likewise believes that a combination of vitamin B deficiency and toxemia is necessary to produce this clinical entity. He advocates, in the absence of any more pronounced indication, the administration of vitamin B in the form of yeast and liver.

In 1937 Forman (45) observed practically complete recovery in a patient following dietary treatment and the administration of dry powdered brewers' yeast. He stated that although the theory of vitamin B_1 deficiency as the etiological agent is probably correct, a large series of cases from a great many clinics must be treated before the theory can be established as fact.

Studies with crystalline vitamin B_1. With vitamin B_1 obtainable in pure crystalline form, the question of whether polyneuritis of pregnancy, like that of beriberi, results solely from a deficiency of vitamin B_1 is nearing solution. Encouraging results have already been secured by Vorhaus, Williams, and Waterman (20), Stähler (46), and Gaehtgens (47). The disease in the three cases studied by Vorhaus, Williams, and Waterman was characterized by tenderness and weakness. All became symptom-free following the oral administration of 10 milligrams of crystalline vitamin B_1 daily. Stähler reported rapid relief of polyneuritis following intramuscular injections of crystalline vitamin B_1 in doses of 4 to 5 milligrams daily. Stähler also recommended accessory treatment with vitamins A and D and the vitamin B complex, although he found that when these sub-

stances were administered separately, they produced no effect on the polyneuritis. Gaehtgens reported definite improvement in a patient following daily subcutaneous injections of "Betaxin" (crystalline vitamin B_1) for eight days. The author recommended treatment of this nature for all severe cases.

The accumulated evidence compels one to regard peripheral neuritis associated with pregnancy as being at least partly nutritional in origin. Furthermore, there is considerable evidence that a deficiency of vitamin B_1 is at least part of the nutritional lack which predisposes to the development of peripheral neuritis in pregnant women.

Diagnosis. The clinical findings in cases of polyneuritis of pregnancy are identical with those of the polyneuritis of beriberi. Hence, the association of pregnancy and polyneuritis in a person whose diet seems inadequate justifies a tentative clinical diagnosis of the polyneuritis of pregnancy.

Prevention and treatment. The prevention and treatment of the polyneuritis of pregnancy are discussed in Chapter VI.

Korsakoff's Syndrome

This syndrome was first described in 1890 by Korsakoff (48) in pointing out the relationship of polyneuritis and psychosis to the vomiting of pregnancy. At the present time, however, the Korsakoff syndrome is nearly always described as a sequela of chronic alcoholic addiction. Clinically, it is usually ushered in by delirium, confabulation, and loss of memory but not by unconsciousness. Polyneuritis is usually associated with this psychosis, and pellagra and other dietary deficiency diseases may or may not be present. It has been the experience of the authors that patients with this syndrome who eat large amounts of a well-balanced diet supplemented with yeast or liver more frequently recover than do those who, for one reason or another, will not eat such diets. There is no conclusive proof at the present time that Korsakoff's syndrome is a deficiency disease or that a lack of vitamin B_1 plays a significant role in its development. It has been advocated that vitamin B concentrates and crystalline vitamin B_1 be administered both orally and parenterally in the severe cases (49), but there is need for critical clinical studies to determine if vitamin B_1 is of specific value.

Pellagra

The pellagra syndrome conspicuously affects the alimentary tract, and the dermal and nervous systems (50, 51, 52). The symptoms arising from involvement of the nervous system, like the other symptoms of pellagra, do not appear in any regular order. They arise mainly from cerebral involvement, combined

degeneration of the spinal cord, and peripheral neuritis. The mental changes may be clinically identical with Korsakoff's psychosis or may manifest themselves as periods of depression and apprehension. The subacute combined degeneration of the spinal cord is characterized by spasticity and ataxia and is clinically identical with the combined system disease associated with pernicious anemia. The peripheral neuritis is clinically indistinguishable from the polyneuritis of beriberi and chronic alcoholism.

In the preceding section it was shown that vitamin B_1 plays a significant role in the development of "alcoholic" polyneuritis. Many of the alcoholic addicts with polyneuritis had pellagra as well. It has been shown recently (10, 12) that "alcoholic" polyneuritis is clinically and pathologically identical with the polyneuritis associated with endemic pellagra (pellagra unassociated with alcoholism). In a still more recent study (53) it was found that the polyneuritis of thirty-one cases of pellagra unassociated with alcoholism disappeared following treatment with a well-balanced diet supplemented with large amounts of the vitamin B complex. It thus seemed evident that polyneuritis associated with endemic pellagra, like that associated with chronic alcoholism, is nutritional in origin. By analogy with the neuritis associated with alcoholism and beriberi, it seemed possible that vitamin B_1 might play an important part in the etiology of the polyneuritis of endemic pellagra.

Spies and Aring (54) have shown that the pain and numbness associated with the peripheral neuritis in six cases of pellagra (two "endemic" and four "alcoholic") remitted promptly following treatment with crystalline vitamin B_1 (Merck). In both of the "endemic" cases and in one of the "alcoholic," nicotinic acid produced healing of the mucous membrane lesions within twenty-four hours, but apparently did not affect the pain and numbness from the peripheral neuritis. These symptoms subsided rapidly, however, following the injection of 50 milligrams of vitamin B_1 on two successive days. These findings suggest that vitamin B_1 should be used in the treatment of the polyneuritis of endemic and so-called "alcoholic" pellagra.

Prevention and treatment. For prevention and treatment of the polyneuritis of pellagra, see Chapter VI.

Sprue

Sprue is a chronic, wasting disease characterized by anemia, diarrhea, and stomatitis. Ashford (55) states that it is common in many tropical countries in which the consumption of meat, dairy products, and green vegetables is low, and that it affects especially those persons coming from northern latitudes who

"cannot eat the native food." The symptomatology of sprue resembles that of pellagra and pernicious anemia more than that of beriberi, though edema of the feet and legs is not uncommon and in a small percentage of cases paresthesias of the extremities are present (56, 57, 58, 59). Symptoms arising from spinal cord lesions are unusual, but involvement of the peripheral nerves has been noted frequently (60, 61). The peripheral neuritis of tropical and nontropical sprue is clinically indistinguishable from that of beriberi, pellagra, or pernicious anemia. It is relieved, like the other symptoms of sprue, by the administration of an adequate diet rich in protein (62, 63, 64, 65, 66), and by injections of liver extract (61, 67). These observations indicate that the involvement of the nervous system in sprue is nutritional in origin. As yet there is no report in the literature as to whether vitamin B_1 plays a specfic role in preventing the development of these neurological changes.

Pernicious Anemia

About 75 per cent of all cases of Addisonian pernicious anemia have involvement of the nervous system (68, 69), and in at least 25 per cent of all cases, neural or mental symptoms are the first indications of the disease. The symptoms arising from involvement of the nervous system are usually characterized by disorders of sensation and tingling of the hands and feet (70, 71, 72). About 10 per cent of all patients having symptoms arising from the nervous system have degeneration of the posterior and lateral column, characterized by spastic ataxia, increased reflexes, positive Babinski sign, and loss of vibration and position sense (56). The peripheral neuritis in patients with pernicious anemia cannot be distinguished pathologically (73) from the peripheral neuritis associated with pellagra, chronic alcoholism, and other conditions described in this chapter.

It has been shown that the progression of the spinal cord and peripheral nerve lesions in pernicious anemia can be averted by the administration of adequate amounts of a crude intramuscular liver extract (69, 74, 75, 76). Liver extracts administered a few years ago were considerably less refined than some that are now being dispensed (77), and it may be that the more refined concentrates now in use will not protect against neural involvement. This point should be determined within the next few years. In general, it is believed that the more crude the liver extract, the greater is the content of vitamin B_1. In 1932 Fouts, Kempf, Greene, and Zerfas (78) reported no improvement of the neurological findings (but these were not peripheral neuritis) in six patients with pernicious anemia following intravenous

injections of a concentrated extract of vitamin B. However, there have been a few cases reported in which the neurological lesions are said to have been cured by means of vitamin B_1 (79, 80), but these cases were uncontrolled, few in number, and do not constitute significant evidence. At the present time the physician will do well to use the forms of liver extract of proved effectiveness in the treatment of spinal cord lesions. Vitamin B_1 preparations are, of course, theoretically valuable accessories and their use should not be denied.

Colitis

For some time it has been recognized that intestinal dysfunction is often associated with malnutrition and deficiency diseases (81, 82, 83). Mackie and Pound (84) have reported that 63 per cent of seventy-five cases of ulcerative colitis showed objective evidence of some deficiency disease. Marks (85) has been able to relieve some of the complaints associated with colitis by means of wheat germ. Brown (86) has reported beneficial results following the administration of large amounts of crystalline vitamin B_1 to cases of ulcerative colitis. The peripheral neuritis associated with colitis is clinically the same as that associated with other conditions described in this chapter. On theoretical grounds it seems likely that a lack of vitamin B_1 plays a definite role in the development of this neuritis, but no conclusive publications on this subject have as yet appeared.

Diabetes

Attempts to explain the high incidence of neurological lesions in diabetes began with the original concept, suggested by Bernard's work, that the nervous lesions were the cause rather than the result of disturbed sugar metabolism. Marshall de Calvi, in 1864, first expressed doubt of the validity of this concept, and since his time many theories have been advanced only to be discarded subsequently. By the end of the first decade of the insulin era, arteriosclerosis and vitamin deficiency were believed by most writers to rank high among the etiological agents responsible for the neurological lesions. Angle (87), in 1928, reported a case of "diabetic tabes" which improved on a diet rich in vitamins. In the following year Minot (4) described two diabetic patients with peripheral neuritis and achylia gastrica who improved slowly by taking large amounts of a yeast concentrate. In 1930 Wechsler (37) and Root and Rogers (88) suggested that on theoretical grounds diabetic neuritis might be caused by a vitamin deficiency.

Since the polyneuritis of diabetes and of beriberi may give

rise to similar or identical symptoms, it was natural that crystalline vitamin B_1 should be given to patients with diabetic neuritis. Published reports to date are surprisingly few, and though suggestive, they are far from conclusive. Vorhaus, Williams, and Waterman (20) included diabetic neuritis in a group of metabolic neuritides which improved following crystalline vitamin B_1 therapy. (See section on carbohydrate metabolism.) Likewise, Sciclounoff and Broccard (21), in 1936, described excellent results in five out of six cases of diabetic neuritis treated with parenteral crystalline vitamin B_1 preparations. Their case reports said little about concurrent diabetic control. One can feel confident that vitamin B_1 deficiency does occur in certain diabetics, but what percentage of the cases of neuritis in diabetics have vitamin B_1 deficiency cannot be estimated with any degree of assurance. Nor can we state whether or not local ischemia resulting from sclerosis of the vessels might predispose to some of the manifestations.

Studies of the lesions show extensive myelin degeneration in the peripheral nerves and their tracts, but the relation of this degeneration to the neuritic symptoms has not been elucidated. Neurological damage in the diabetic is suspected or diagnosed because of pain, paresthesias, or altered reflexes which often are associated with hyperesthesia, anesthesia, proprioceptive disturbances, sphincter impairment, abnormal pupillary responses, or ataxia. The problems involved in any clinical classification have been emphasized by Jordan (89) in his comprehensive review. When a diabetic presents symptoms or signs suggestive of neurological change, other conditions producing similar manifestations must be controlled before we can evaluate the findings. At the present time it seems that the prophylaxis against neuritis in diabetic patients, in addition to painstaking control of the diabetes, requires prescriptions of diet adequate in the antineuritic vitamin. Sindoni (90) has shown that several typical diabetic diets are actually deficient in this vitamin. The diets used in the clinical management of diabetics in many hospitals are on the borderline of normal requirements and would be inadequate in the event of any additional requirement.

Since diabetes and vitamin B_1 deficiency are common, the two will sometimes occur by coincidence in the same person. At the present time, information is inadequate to state that the majority of cases of diabetic neuritis are caused by an inadequate amount of vitamin B_1. The following theoretical considerations, however, favor the hypothesis that vitamin B_1 deficiency may play a part in the etiology of these changes : 1. The close relationship between vitamin B_1 and carbohydrate metabolism which is discussed elsewhere in this book (see Chapters XXI and XXVI). 2. The similarity of the clinical and neuro-

pathologic pictures between diabetic neuritis and the neuritis associated with other diseases which are known to be due to a lack of vitamin B_1. 3. The realization that many diabetics subsist on diets which are low in vitamins.

Prevention and treatment. The prevention and treatment of diabetic neuritis are discussed in Chapter VI.

Tuberculosis

In descriptions of tuberculosis (91), one occasionally sees a general statement to the effect that peripheral neuritis occurs in the late stages of this disease. Some investigators (3, 7, 92, 93) have suggested that the neuritis is associated with the accompanying emaciation. Schiro, Aring, and Spies (94) showed that the clinical and pathological manifestations of neuritis in three persons with tuberculosis were identical with those of the peripheral neuritis associated with the other diseases referred to in this chapter. The neuritis in these three patients developed while the patients were in the hospital and disappeared following the administration of large amounts of yeast and a well-balanced diet. These studies suggest that dietary deficiency played a definite role in the development of the polyneuritis.

Porphyrinuria

It has been observed for some time that increased quantities of porphyrin may be excreted in the urine in febrile conditions, liver diseases, anemia, and in poisoning by sulphonal, veronal, trional, lysol, chloroform, salvarsan, zinc, acetanilid, tin, and lead (95, 96, 97, 98). Some writers (99, 100) regard this finding as a simple increase in the amount of normal porphyrin excretion resulting from the underlying disturbances in metabolism. In recent years it has been shown (101, 102, 103) that a number of the metabolic and deficiency diseases which are accompanied by peripheral neuritis also are associated with porphyrinuria. It seems likely that porphyrinuria occurs as a physiological process and that the amount excreted may be increased in certain diseases and intoxications. Very rarely porphyrinuria may be the chief symptom of a characteristic clinical syndrome of unknown etiology. When this disease affects the nervous system, the first symptom is severe and constant pain in the extremities. This is usually accompanied by muscular weakness and paralysis of the lower motor neuron type. Pathological examination shows widespread degeneration of the myelin sheaths in the peripheral nerves and spinal cord (95). At present there is little information as to whether vitamin B_1 bears a relationship to any of the neuritides associated with porphyrinuria.

Senility

Senility is frequently associated with a change in dietary habits, decreased appetite, loss of teeth, and sclerosis of the arteries. Elderly persons with cachexia frequently have tender feet and at times have easily demonstrable peripheral neuritis. This peripheral neuritis is similar clinically to the peripheral neuritis of beriberi. It is possible that the peripheral neuritis associated with senility and cachexia may be due to a deficiency of some nutritional factor such as vitamin B_1.

Tumors and Cirrhosis of the Liver

Tumors and cirrhosis of the liver in the later stages characteristically produce cachexia. The cancerous or cirrhotic process may interfere directly with the appetite or may interfere with digestion and assimilation. Not infrequently, persons with cachexia associated with malignant tumors or with cirrhosis have peripheral neuritis, and this type of neuritis may be caused by inadequate nutrition. Heiman (104) is of the opinion that vitamin B_1 may play some part in the development of this neuritis. Patek (105) has shown that certain persons with cirrhosis are benefited following the administration of the vitamins.

BIBLIOGRAPHY

1. Lettsom, J. C.: Some Remarks on the Effects of Lignum Quassiae Amarae. Mem. M. Soc. London *1*, 128, 1779–1787.
2. Jackson, J.: On a Peculiar Disease Resulting from the Use of Ardent Spirits. New England J. Med. and Surg. *11*, 351, 1822.
3. Shattuck, G. C.: The Relation of Beri-beri to Polyneuritis from Other Causes. Am. J. Trop. Med. *8*, 539, 1928.
4. Minot, G. R.: Some Fundamental Clinical Aspects of Deficiencies. Ann. Internal Med. *3*, 216, 1929.
5. Minot, G. R.: The Treatment of Anemia, with Comments on Food Deficiency and Its Relation to the Nervous System. Tr. A. Neurol. A., p. 329, 1931.
6. Meyer, A.: Ueber das Vorkommen von B-Avitaminose unter den hiesigen Lebensbedingungen. Schweiz. med. Wochschr. *62*, 1243, 1932.
7. Wechsler, I. S.: Etiology of Polyneuritis. Arch. Neurol. & Psychiat. *29*, 813, 1933.
8. Minot, G. R., Strauss, M. B., and Cobb, S.: "Alcoholic" Polyneuritis; Dietary Deficiency as a Factor in Its Production. New England J. Med. *208*, 1244, 1933.

9. Bloomfield, A. L., and Polland, W. S.: Gastric Anacidity: Its Relation to Disease. The Macmillan Co., New York, 1933.
10. Spies, T. D., and DeWolf, H. F.: Observations on the Etiological Relationship of Severe Alcoholism to Pellagra. Am. J. Med. Sci. *186,* 521, 1933.
11. Strauss, M. B.: The Etiology of "Alcoholic" Polyneuritis. Am. J. Med. Sc. *189,* 378, 1935.
12. Blankenhorn, M. A., and Spies, T. D.: Prevention, Treatment and Possible Nature of the Peripheral Neuritis Associated with Pellagra and Chronic Alcoholism. Tr. Assoc. Am. Physicians *50,* 164, 1935.
13. Cowgill, G. R.: The Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
14. Jolliffe, N., Colbert, C. N., and Joffe, P. M.: Observations on the Etiologic Relationship of Vitamin B (B_1) to Polyneuritis in the Alcohol Addict. Am. J. Med. Sc. *191,* 515, 1936.
15. Jolliffe, N., and Colbert, C. N.: The Etiology of Polyneuritis in the Alcohol Addict. J.A.M.A. *107,* 642, 1936.
16. Jolliffe, N., and Goodhart, R.: Beriberi in Alcoholic Addicts. (In press.)
17. Romano, J.: Deficiency Syndromes Associated with Chronic Alcoholism. A Clinical Study. Am. J. Med. Sci. *194,* 645, 1937.
18. Williams, R. R., Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin. J. Am. Chem. Soc. *56,* 1187, 1934.
19. Williams, R. R., and Cline, J. K.: Synthesis of Vitamin B_1. J. Am. Chem. Soc. *58,* 1504, 1936.
20. Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1. Experimental and Clinical Observations. J.A.M.A. *105,* 1580, 1935.
21. Sciclounoff, F., and Broccard, R.: La Vitamine B_1 dans le Traitement des Polynévrites. Schweiz. med. Wochschr. *66,* 985, 1936.
22. Neumann, H.: Erfahrungen mit Betaxin (Vitamin B_1-Präparat) in der Neurologie. Münch. med. Wochschr. *82,* 1959, 1935.
23. Russell, W. R.: The Parenteral Administration of Vitamin B_1 in the Treatment of Polyneuritis and Other Conditions. Edinburgh M. J. *43,* 315, 1936.
24. Desrochers, G., and Larue, G. H.: Acquisitions Récentes sur le Traitement des Polynévrites Alcooliques; Présentation d'un Cas. Laval. méd. *2,* 81, 1937.
25. Goodhart, R., and Jolliffe, N.: Effects of Vitamin B (B_1) Therapy on the Polyneuritis of Alcohol Addicts. J.A.M.A. 110, 414, 1938.
26. Berkwitz, N. J., and Lufkin, N. H.: Toxic Neuronitis of Pregnancy; A Clinicopathological Report. Surg., Gynec., and Obst. *54,* 743, 1932.

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27. Plass, E. D., and Mengert, W. F.: Gestational Polyneuritis. J.A.M.A. *101*, 2020, 1933.
28. Whitfield, D. W.; Peripheral Neuritis Due to the Vomiting of Pregnancy. Lancet, I, 627, 1889.
29. Tuilant, A.: De la Névrite Puerpérale. Paris, 1891. Quoted from Berkwitz and Lufkin (26).
30. Hoesslin, R.: Die Schwangerschaftslaehmungen der Muetter. Arch. f. Psychiat. *38*, 730, 1903; *40*, 445, 1905.
31. Albeck-Aarhus: Über Polyneuritis gravidarum. Arch. f. Gynäk. *117*, 41, 1922.
32. Ely, F. A.: Memory Defect of Korsakoff's Type, Observed in Multiple Neuritis Following Toxemia of Pregnancy. J. Nerv. & Ment. Dis. *56*, 115, 1922.
33. Weill-Hallé, B., and Layani, F.: Polynévrite et Syndrome de Korsakoff au Cours de la Gestation. Bull. et mém. Soc. méd. d. hôp. de Paris *51*, 145, 1927.
34. Ledoux, E.: Polynévrite Gravidique avec Syndrome de Korsakow. Presse Méd. *37*, 516, 1929.
35. Dupouy, R., and Courtois, A.: Des Psychoses, Gravidiques et en Particulier de la Psychopolynévrite. Syndrome de Korsakoff. Encéphale *25*, 284, 1930.
36. Hofmann, J.: Beriberi in Chinese Women and Its Relation to Childbearing. The China Med. J. *38*, 987, 1924.
37. Wechsler, I. S.: Unrecognized Cases of Deficiency Polyneuritis (Avitaminosis?). Preliminary Report. Med. J. and Rec. *131*, 441, 1930.
38. Theobald, G. W.: The Causation of Eclampsia; Observations and Experiments. Lancet I, 1115, 1930.
39. Strauss, M. B., and McDonald, W. J.: Polyneuritis of Pregnancy; A Dietary Deficiency Disorder. J.A.M.A. *100*, 1320, 1933.
40. Luikart, R. R.: Avitaminosis as a Likely Etiologic Factor in Polyneuronitis Complicating Pregnancy, with Report of a Case. Am. J. Obst. and Gynec. *25*, 810, 1933.
41. Fouts, P. J., Gustafson, G. W., and Zerfas, L. G.: Successful Treatment of a Case of Polyneuritis of Pregnancy. Am. J. Obst. and Gynec. *28*, 902, 1934.
42. Theobald, G. W.: Neuritis in Pregnancy Successfully Treated with Vitamin B_1. Lancet, I, 834, 1936.
43. Bingham, A. W.: Polyneuritis of Pregnancy: A Report of Four Cases. Am. J. Obst. and Gynec. *32*, 144, 1936.
44. Ford, R. K.: Polyneuritis of Pregnancy. J. Obst. and Gynec., Brit. Emp. *42*, 641, 1935.
45. Forman, G. W.: Polyneuritis of Pregnancy: Treatment with Vitamin B_1. Case Report. J. Missouri M. A. *34*, 197, 1937.
46. Stähler, F.: B_1-Hypovitaminosen in der Schwangerschaft. Münch. med. Wochschr. *84*, 327, 1937.

47. Gaehtgens, G.: Ueber die Traumatisch Bedingte Wochenbettneuritis. Münch. med. Wochschr. *83*, 720, 1936.
48. Korsakoff, S.: Eine psychische Störung combinirt mit multipler Neuritis (Psychosis Polyneuritica seu Cerebropathia Psychica Toxaemica). Allg. Ztschr. f. Psychiat., Berlin, *46*, 475, 1889–90.
49. Weiss, S.: Cecil's Textbook of Medicine, 4th Ed. W. B. Saunders Co., Philadelphia., 1937.
50. Sebrell, W. H.: Pellagra. Tice's Practice of Medicine. W. F. Prior Company, Hagerstown, Maryland. *9*, 205, 1933.
51. Taylor, F. R.: Pellagra. Oxford Medicine *4*, 307, 1929.
52. Spies, T. D.: Pellagra. Cecil's Textbook of Medicine, 4th Ed. W. B. Saunders Co., Philadelphia, 1937.
53. Spies, T. D., Chinn, A. B., and McLester, J. B.: Severe Endemic Pellagra. A Clinical Study of Fifty Cases with Special Emphasis on Therapy. J.A.M.A. *108*, 853, 1937.
54. Spies, T. D., and Aring, Charles: The Effect of Vitamin B_1 on the Peripheral Neuritis of Pellagra, J.A.M.A. *110*, 1081, 1938.
55. Ashford, B. K.: Sprue. Ann. Clin. Med. *4*, 13, 1925.
56. Castle, W. R., and Minot, G. R.: Pathological Physiology and Clinical Description of the Anemias. Oxford University Press, New York, 1936.
57. Reed, A. C.: Sprue. A Clinical Summary. Am. J. Trop. Med. *16*, 499, 1936.
58. McLester, J. S.: Clinical Syndromes That Include Achlorhydria. J.A.M.A. *95*, 719, 1930.
59. Castle, W. B., and Rhoads, C. P.: Observations on the Etiology and Treatment of Sprue in Puerto Rico. Trans. Assn. Am. Physicians *47*, 245, 1932.
60. Hanes, F. M.: Personal communication.
61. Fairley, N. H.: Personal communication.
62. Mackie, F. P., and Fairley, N. H.: Sprue: Its Applied Pathology, Biochemistry and Treatment. Proc. Roy. Soc. Trop. Med. and Hyg. *24*, 131, 1930.
63. Mackie, T. T.: Non-tropical sprue. Med. Clinics N. Amer. *17*, 165, 1933.
64. Fairley, N. H.: Intestinal Absorption in the Steatorrhoeas. Lancet, I, 1298, 1936.
65. Hanes, F. M.: Sprue. Cecil's Textbook of Medicine, 4th Ed. W. B. Saunders Company, Philadelphia., 1937.
66. Musser, J. H.: Sprue. Internal Medicine; Its Theory and Practice. Lea and Febiger, Philadelphia, 1932.
67. Rhoads, C. P., and Miller, D. K.: Intensive Liver Extract Therapy of Sprue. J.A.M.A. *103*, 387, 1934.
68. Smithburn, K. C., and Zerfas, L. G.: The Neural Symptoms and Signs in Pernicious Anemia. Arch. Neurol. and Psychiat. *25*, 1100, 1931.

69. Baker, B. M., Jr., Bordley, J., III, and Longcope, W. T.: The Effect of Liver Therapy on the Neurologic Manifestations of Pernicious Anemia. Am. J. Med. Sci. *184*, 1, 1932.
70. Lurie, L. A.: Pernicious Anemia with Mental Symptoms: Observations on Varying Extent and Probable Duration of Central Nervous System Lesions in Four Necropsied Cases. Arch. Neurol. and Psychiat. *2*, 67, 1919.
71. McAlpine, D.: A Review of the Nervous and Mental Aspects of Pernicious Anaemia. Lancet, II, 643, 1929.
72. Greenfield, J. G., and O'Flynn, E.: Subacute Combined Degeneration and Pernicious Anaemia. Lancet, II, 62, 1933.
73. Greenfield, J. G., and Carmichael, E. A.: The Peripheral Nerves in Cases of Subacute Combined Degeneration of the Cord. Brain *58*, 483, 1935.
74. Strauss, M. B., Solomon, P., Schneider, A. J., and Patek, A. J., Jr.: Subacute Combined Degeneration of the Spinal Cord in Pernicious Anemia: The Complete Arrest of the Lesion with Parenteral Liver Therapy. J.A.M.A. *104*, 1587, 1935.
75. Ungley, C. C., and Suzman, H. M.: Subacute Combined Degeneration of the Cord: Symptomatology and Effects of Liver Therapy. Brain *52*, 271, 1929.
76. Goldhamer, S. M., Bethell, F. H., Isaacs, R., and Sturgis, C. C.: The Occurrence and Treatment of Neurologic Changes in Pernicious Anemia. J.A.M.A. *103*, 1663, 1934.
77. Minot, G. R., and Castle, W. B.: Year Book of General Medicine, p. 352, 1937.
78. Fouts, P. J., Kempf, G. F., Greene, J. A., and Zerfas, L. G.: Vitamin B Intravenously for the Treatment of Neurological Changes in Pernicious Anemia. J. Indiana State Med. Assn. *25*, 448, 1932.
79. Lasch, F.: Ein Erfolgreich mit Vitamin B_1 Behandelter Fall von Schwerer Funikulärer Myelose. Münch. med. Wochschr. *83*, 1390, 1936.
80. Bergel, A.: Ein Behandlungserfolg bei Funikulärer Myelose mittels Vitamin B_1 (Betabion). Deut. med. Wochschr. *62*, 1643, 1936.
81. Miller, D. K., and Rhoads, C. P.: The Effect of Liver Extract on the Small Intestine of Patients with Sprue. Am. J. Med. Sci. *191*, 453, 1936.
82. Strauss, M. B.: The Role of the Gastro-intestinal Tract in Conditioning Deficiency Disease. J.A.M.A. *103*, 1, 1934.
83. Eusterman, G. B., and O'Leary, P. A.: Pellagra Secondary to Benign and Carcinomatous Lesions and Dysfunction of the Gastro-Intestinal Tract: Report of Thirteen Cases. Arch. Internal Med. *47*, 633, 1931.
84. Mackie, T. T., and Pound, R. E.: Changes in the Gastro-intestinal Tract in Deficiency States, with Special Reference to the

Small Intestine: A Roentgenologic and Clinical Study of Forty Cases. J.A.M.A. *104,* 613, 1935.
85. Marks, H. E.: Chronic Vitamin B Deficiency: A Clinical Study. M. J. and Rec. *135,* 231, 1932.
86. Brown (1936). Cited by Stepp, Kühnau, and Schroeder. Die Vitamine. Ferdinand Enke. 1937.
87. Angle, F. E.: Tabes Diabetica; Report of a Case. U. S. Nav. Med. Bull. *26,* 81, 1928.
88. Root, H. F., and Rogers, M. H.: Diabetic Neuritis with Paralysis. New England J. Med. *202,* 1049, 1930.
89. Jordan, W. R.: Neuritic Manifestations in Diabetes Mellitus. Arch. Int. Med. *57,* 307, 1936.
90. Sindoni, A., Jr.: Vitamin Deficiency in Prescription Diets of Diabetics: Study into Relationship of Diet Deficiency to Symptomatology as Observed in 85 Diabetics with Previous Dietary Treatment. Am. J. Digest. Dis. & Nutrition *3,* 759, 1936.
91. Landis, H. R. M.: Tuberculosis. Oxford Medicine *5,* 259.
92. Cobb, Stanley: A Preface to Nervous Disease. William Wood and Company, Baltimore, 1937.
93. Hasselbach, F.: Polyneuritiden bei Lungen- und Dermtuberkulose, ihre Beziehung zur B_1-Mangelkrankheit und zur Frage der Polyavitaminosen. Deut. med. Wochschr. *63,* 1150, 1937.
94. Schiro, H., Aring, C., and Spies, T. D.: Unpublished observations.
95. Mason, V. R., Courville, C., and Ziskind, E.: The Porphyrins in Human Disease. Medicine *12,* 335, 1933.
96. Watson, C. J.: Concerning the Naturally Occurring Porphyrins. IV. The Urinary Porphyrins in Lead Poisoning as Contrasted with That Excreted Normally and in Other Diseases. J. Clin. Invest. *15,* 327, 1936.
97. Dobriner, K.: Urinary Porphyrins in Disease. J. Biol. Chem. *113,* 1, 1936.
98. Watson, C. J.: Concerning the Naturally Occurring Porphyrins. I. The Isolation of Coproporphyrin I from the Urine in a Case of Cinchophen Cirrhosis. J. Clin. Invest. *14,* 106, 1935.
99. Günther, H.: Hämatoporphyrie. Deut. Arch. f. klin. Med. *105,* 89, 1911.
100. Garrod, A. E.: Inborn Errors of Metabolism. Henry Frowde, London, p. 136, 1923.
101. Beckh, W., Ellinger, P., and Spies, T. D.: Porphyrinuria in Pellagra. Quart. J. Med. *6,* 305, 1937.
102. Watson, C. J.: Concerning the Naturally Occurring Porphyrins. V. Porphyrins of the Feces. J. Clin. Invest. *16,* 383, 1937.
103. Fischer, H.: Über Blut, Blatt u. Gallenfarbstoff. Oppenheimer's Handb. der Biochem. des Menschen u. der Tiere. Zweite Auflage, Ergänzungsband, G. Fischer, Jena, p. 72, 1930.

104. Heiman, M.: Zur Vitaminbehandlung der Nervenkrankheiten; Klinik und Therapie des Vitamin B_1. Klin. Wochschr. *16*, 1076, 1937.

105. Patek, A. J.: Treatment of Alcoholic Cirrhosis of the Liver with High Vitamin Therapy. Proc. Soc. Exper. Biol. and Med. *37*, 329, 1937.

CHAPTER IV

PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY OF VITAMIN B_1 DEFICIENCY IN ANIMALS AND HUMAN BEINGS

Pathology of Vitamin B_1 Deficiency in Animals

The majority of investigators regard experimental vitamin B_1 deficiency as a disease analogous to human beriberi, but the manifestations of the two are often different. Experimental beriberi develops rapidly and resembles infantile beriberi more closely than any other type. It is frequently difficult to relate the findings of experimental vitamin B_1 deficiency to those of human beriberi because of the differences in species and in the diets of experimental animals and those of human beings. Some investigators are of the opinion that the diets of persons who develop beriberi are often deficient in factors other than the vitamin B complex. Morphological and histo-pathological studies have been distinctly unsatisfactory in explaining the precise role of vitamin B_1; however, the following findings seem to be of importance in spite of the lack of satisfactory methods of study.

1. **The nervous system lesions.** Degeneration of the nervous system occurs in most, if not all, species suffering from prolonged inadequacy of vitamin B_1. In the rat and in the pigeon, continued depletion of vitamin B_1 results in death. As early as 1897 Eijkman (1) showed that degenerative changes were present in the peripheral nerves and anterior horn cells of fowls with experimental beriberi. He considered these findings analogous to those of human beriberi. In recent years, however, there has been a strong tendency to consider many of the pathological changes of experimental beriberi the result of starvation rather than the result of a vitamin B_1 deficiency. Particularly significant, in support of this idea, is the general opinion among investigators that one of the earliest results of a deprivation of vitamin B_1 is failure of appetite. In 1911 Chamberlain, Bloombergh, and Kilbourne (2) demonstrated that fowls dying from starvation had degeneration of the peripheral nerves which could not be distinguished readily from the degeneration found in polyneuritis gallinarum induced by an exclusive rice diet. In 1937 Davison and Stone (3) concluded that "the pathological

changes in the nervous system in animals suffering from inanition are essentially the same as those deprived of vitamin B_1 or both vitamins B_1 and B_2. If anything, the changes in the peripheral nerves in animals subjected to inanition are more marked." Recently, Vedder and Chinn (4) compared the brachial and sciatic nerves of rats subjected to complete starvation, rats completely starved but given 3 milligrams of crystalline vitamin B_1 orally each day, and rats given a diet deficient in vitamin B_1. Myelin degeneration was seen in the peripheral nerves of all rats of all groups. No appreciable difference in the extent of this degeneration was noted in the three groups. Sebrell and Elvove (5) have shown that an inadequate amount of vitamin B_1 added to the vitamin B_1 free diet of rats makes the polyneuritis more severe than if no vitamin B_1 is added. Vedder (6) has recently stated: "It must be admitted that rats starved to death showed definite degenerative changes in the nerves of the same general character as those found in polyneuritis. Nor are these degenerative changes eliminated when such starving animals are given adequate amounts of vitamin B_1. The degenerative changes are, as a rule, quite slight and consist of occasional minute droplets taking the black stain and, according to my belief, are not as extensive as the changes found in the polyneuritis caused by feeding a diet deficient in vitamin B_1." It is essential that further studies on the physiological action of vitamin B_1 be made before we can determine how definite a role inanition plays in the development of polyneuritis in vitamin B_1 deficient animals.

The lesions occurring in experimental polyneuritis appear identical to those occurring in human beriberi. Human beings, however, often develop the lesions while well nourished. From studies on human beings it is thought by the authors that diets deficient in other essential factors increase the requirement for vitamin B_1 and that this substance is not stored in the body to the extent that we previously thought. It would seem, therefore, that any of these factors, in addition to a lack of vitamin B_1, might play a significant role in the development of human beriberi.

Experimental polyneuritis in fowls usually is comparable to dry beriberi in man. In the experimental animal, as in the human being with vitamin B_1 deficiency, most degeneration is in the peripheral nerves, the sciatic nerves being particularly affected. The excellent description by Vedder and Clark (7) shows that practically every fiber has some evidence of degeneration, though the extent varies greatly in the fibers of the same nerve. The myelin is degenerated, and there is swelling of the nerve sheath in 10 to 15 per cent of the fibers. Vedder describes the earliest stage of myelin degeneration as slight swelling of the medullary

sheath and a tendency toward segmentation at the circumference. These changes are generalized, affecting the peripheral nerves, the ventral and dorsal nerve roots, all tracts of the spinal cord, the medulla, pons, midbrain, and internal capsule. There is also degeneration of the nerve cells. These changes in the nervous system appear similar to those already described in human beriberi. It is thought, however, that in order to produce lesions similar or identical to those appearing in human beings, some vitamin B_1 must be fed to the experimental animal in order to prevent early death (8).

Vitamin B_1 deficiency in the dog is often characterized by spastic phenomena similar to those so often seen in human beings with a vitamin B_1 deficiency (8). In the dog the lesions of the nervous system are variable in location, order of appearance, and severity. One of the most amazing and least understood facts is that the clinical manifestations, except in the terminal stages, show dramatic and rapid improvement within a few hours, and yet complete recovery may be delayed. Similarly, the histopathological changes in the nervous system appear after the symptoms develop, and persist after symptomatic recovery is under way (9).

2. **The cardiovascular system, edema, and serous effusions.** The serous effusions and cardiac hypertrophy described in human beriberi are rarely reported in the experimental deficiencies. McCarrison (10), however, succeeded in producing edema and enlarged hearts, in addition to polyneuritis, in a large number of pigeons by feeding them diets similar to those used by the natives of India who develop beriberi. These diets, in addition to rice, include dal (legumes) and other antineuritic foods. The heart in experimental vitamin B_1 deficiency is often atrophic. Bradycardia is so commonly found in vitamin B_1 deficient rats that it is now used as a method of assay for this vitamin. The observations on the cardiovascular system of animals on vitamin B_1 deficient diets are of great interest in view of the recent clinical work of Keefer (11), Feil (12), and Weiss and Wilkins (13), discussed later in this chapter.

Edema and serous effusions are rarely seen in experimental vitamin B_1 deficiency unless there is also a lack of proteins in the diet. It has been shown by Frisch, Mendel, and Peters (14) that rats restricted to diets low in protein regularly develop serum protein deficiency.

3. **The gastro-intestinal tract.** Comprehensive investigations have been made on the effect of vitamin B_1 deficiency on the gastro-intestinal tract. Karr (15) and Cowgill (16) emphasized the fact that lack of vitamin B_1 decreases the desire for food. Cowgill and Gilman (17) showed that hypochlorhydria and achlorhydria frequently develop in dogs restricted to a deficient

diet. "Dystrophy" of the intestine was reported by Abderhalden (18), atony by Rowlands and Browning (19), and degeneration of Auerbach's plexus by McCarrison (20). Eddy and Dalldorf (8) were unable to demonstrate significant lesions in the neural plexus in polyneuritic animals. It seems evident, therefore, that the lesions described by McCarrison are no more constant than are other lesions of the nervous system. McCarrison (20), Dalldorf and Kellogg (21), and Sure and Thatcher (22) have described erosions and ulcers of the stomach. The functional disturbances of the gastro-intestinal tract, however, are much more severe than can be accounted for on the basis of demonstrable anatomical change. The gastric symptomatology of experimental vitamin B_1 deficiency closely resembles the symptomatology of infantile beriberi.

4. **Internal secretions.** Marked hypertrophy of the adrenals is a characteristic finding of experimental vitamin B_1 deficiency. McCarrison (23) has described an increased output of epinephrin, but Kon and Drummond (24) found the amount of epinephrin in the experimental pigeon to be normal. Enlargement of the adrenals is confined to birds and is not observed as the usual thing in either rats or human beings restricted to a diet deficient in vitamin B_1. The Islands of Langerhans are hypertrophic (25, 26), and there is hyperglycemia in fowls deprived of vitamin B_1 (see Chapter XXV). Loss of libido is described in the male animals and prompt suppression of the follicular function of the ovaries in the female. Evans and Bishop (27) have shown that suppression of the follicular function is an early effect of vitamin B_1 deficiency, and Ueno (28) has been able to restore and maintain normal menstrual rhythm by administering follicular and anterior pituitary hormone to vitamin B_1 deficient animals. The thymus, pituitary, thyroid, and liver are atrophic. While we have few facts concerning the relationship between vitamin B_1 and the glands of internal secretion, the relationship seems close indeed.

In summary, it seems fair to state that the present morphological and histological methods yield little to explain the mode of action of vitamin B_1 in either the experimental animal or in human beings. The histo-pathological findings, however, are similar in experimental and human beriberi, and in both, the functional disturbances precede any demonstrable anatomical changes.

Pathology of Vitamin B_1 Deficiency in Human Beings

The pathological changes arising as a result of an inadequate amount of vitamin B_1 in human beings are constant, irrespective of whether the person died from uncomplicated beriberi or

whether he had vitamin B_1 deficiency associated with diseases such as chronic alcoholic addiction, pregnancy, Korsakoff's syndrome, pellagra, sprue, pernicious anemia, colitis, diabetes, tuberculosis, porphyrinuria, senility, malignancy, and cirrhosis. Hence, the authors regard the polyneuritis arising from a deficiency of vitamin B_1 as beriberi, even if another disease condition is present.

Any classification of deficiency diseases, from the standpoint of pathology, is unsatisfactory. While it is not within the scope of this book to describe the general pathology of these various diseases, it must be frankly admitted that in many instances pathologists are unable to determine whether death was due to beriberi, sprue, pellagra, pernicious anemia, celiac disease, chronic alcoholism, or some other closely related disease. This difficulty can be explained in part by the fact that the distribution of lesions within the body, rather than the nature of the etiological agent, often determines the alterations found at postmortem. Another source of difficulty is the frequent occurrence of multiple deficiencies in human beings. When sufficient knowledge becomes available, the classification of these syndromes will be based upon a broader understanding of the lacking chemical substance, the predisposing conditions, and the associated pathological processes.

The pathogenesis of vitamin B_1 deficiency is particularly difficult to determine because the reactions produced in the nervous system by a number of etiological agents are similar. Some of the limitations in our knowledge of the pathology of vitamin B_1 deficiency will be overcome when observations are made which correlate the reactions of the entire nervous system with those in other affected organs of the body. Additional information might be added by applying modern neuro-histological staining methods to nerve structures other than those of the central nervous system and the large nerve trunks.

Despite our limited knowledge of the fundamental pathology of vitamin B_1 deficiency, one can often correlate the clinical course of beriberi with the findings at postmortem. A person dying from acute beriberi is usually not emaciated. The heart in such cases is strikingly enlarged. Patients with chronic beriberi are generally emaciated and usually die from some coexisting disease, usually an infectious one. Complete pathological studies of persons with beriberi demonstrate that multiple neuritis is the most characteristic lesion. In addition to varying degrees of degeneration of the nervous system, changes in the heart, and edema and anasarca may be present. These findings will be discussed under those three headings.

1. **The nervous system.** The most constant and striking symptoms of a vitamin B_1 deficiency arise from degeneration in

the nervous system. There is unusual conformity between the anatomical location of the nervous lesions and the clinical manifestations. The nerves supplying the lower extremities are most commonly affected, but frequently there is degeneration of the cranial and solar plexuses. In the so-called dry form of beriberi there is always polyneuritis in the lower extremities. In the average case early degeneration occurs in the brachial nerves at about the same time that late changes appear in the sciatic nerves. It is characteristic that the distal portions of the nerves are first and most seriously altered. In far-advanced cases all nerves of the body may be involved. All observers (29, 30) agree that there is microscopic evidence of degeneration in the peripheral nerves which may vary from slight alteration to complete disappearance of the myelin and axis cylinders.

Most observers have not reported "naked eye" changes in the brain, peripheral nerves, or spinal cord. Bentley (31), however, has described nineteen necropsies in which he observed congestion and softening of the brain, the spinal cord, and meninges. He stated that the lesions were so large that the cord was soft and diffluent with hemorrhages and edema. Degeneration of the cells of the brain and spinal cord has been shown by the use of the Nissl stain or other cell stains. Disorganization of the cell content and rupture of the membrane have been demonstrated, but it cannot be shown by histological methods whether or not the cell is dead or is still capable of recovery.

The central and peripheral nervous systems in infants are less severely affected by thiamin deficiency than are those of adults.

2. **The heart.** The most common cause of sudden death from vitamin B_1 deficiency is acute cardiac failure. The classical picture at postmortem is a dilated and hypertrophied heart. The right auricle and right ventricle are conspicuously enlarged; the chambers are filled with blood and the valves are normal. At times, when the enlargement of the right auricle and ventricle is pronounced, the left heart may be small. In such instances the walls are "paper thin" as a result of the dilatation of the right auricle and conus arteriosus. Chronic passive congestion of the liver, spleen, kidneys, and intestines is nearly always present, and pulmonary edema occurs in at least 50 per cent of the cases.

The weight of the heart in the classical case of beriberi is increased. Ellis (32), in a study of 125 necropsies of beriberi, found the average weight of the heart to be 13.37 oz. (427.8 grams), whereas the heart in 204 patients who died of other diseases averaged less than 9 oz. (288 grams). Likewise, Japanese workers (33) have found that the average weight of the heart in patients with beriberi is above the maximum weight of the

normal heart. McLaughlin and Andrews (34) described similar findings in infantile beriberi. In the average case the heart weighed 34.1 grams, while in other diseases of infants the average heart weighed 20 grams. These investigators state that the gross criteria essential for the postmortem diagnosis of infantile beriberi are as follows: 1, dilatation and hypertrophy of the right heart; 2, congestion of the viscera; 3, anasarca; 4, absence of other findings to account for death. They believe that the most striking and constant changes are found in the right heart where the musculature is hypertrophied and forms the major portion of the organ. They state that the trabeculae and papillary muscles are prominent and that the cavity is dilated. The walls of the right ventricle measured from 5 to 7 mm. in thickness, while the left measured only from 3 to 5 mm.

The reports of Keefer (11) and Yang and Huang (35) indicate that right-sided dilatation is not an essential feature of the beriberi heart as seen in China. Weiss and Wilkins (13) recently described in Boston, Massachusetts, thirty-five cases of heart disease which they thought due to deprivation of vitamin B_1. They stressed the differences between the characteristics of cardiovascular dysfunctions due to a deficiency of vitamin B_1, as observed in Boston, and those described for the classical beriberi heart in the Orient. Some of the cases described by Weiss and Wilkins had small hearts; some had less right- than left-sided failure; and others had left-sided failure alone. No satisfactory anatomical explanation of the cardiac lesions of beriberi exists, and microscopic examination reveals little pertinent information. There is, however, an increase of intra- and intercellular fluid in some cases.

3. **Edema, serous effusions, and other findings.** Edema and serous effusions are found commonly in cases of acute beriberi. They occur less frequently in chronic cases. The edema is most noticeable in the legs and thighs, and although it may become generalized, it rarely affects the face. Following postmortem incision of the wet case, large amounts of a clear, greenish-yellow serous fluid pour from the subcutaneous tissue. This edema is often associated with effusions into the pericardium, the pleurae, and the peritoneal cavity. Edema of the lungs occurs in fully 50 per cent of the cases.

There is great reduction, if not complete absence, of the subcutaneous, retroperitoneal, and epicardial fat in the chronic cases of beriberi. The affected muscles, particularly in the feet and legs, are atrophied. Microscopic examination shows atrophy of the muscle cells, fatty degeneration, cloudy swelling, and loss of striation. In those cases having edema the fluid can be recognized in the muscle preparations. The various organs of the body are often atrophied.

Beriberi of infants is nearly always associated with edema and serous effusions.

It can be seen from this description that postmortem studies of beriberi have contributed relatively little toward an understanding of the pathogenesis of the disease. The criteria for diagnosis are distinctly unsatisfactory and depend essentially upon the association of a rather characteristic cardiac dilatation of unknown cause, and degeneration of the peripheral nerves. In view of these uncertain criteria, subclinical forms will almost certainly pass unrecognized.

Pathological Physiology

The mechanism of neuritis in vitamin B_1 deficiency. All species deprived of vitamin B_1 over a period of time develop neural degeneration. There is a striking correlation between the degeneration of the nerves and the clinical manifestations. Almost invariably the terminal portions of the nerves are most seriously affected, and the symptoms accordingly are most severe in the distal portions of the extremities. Since the legs are usually first affected by the neuritis of beriberi, it follows that the degeneration is most marked in the sciatic nerves and its branches, and, in addition, that the manifestations here are the last to disappear. The recognizable changes in the nerves, however, probably appear relatively late in the disease process and persist after symptomatic recovery has been initiated. It has been observed that following adequate treatment, the first stages of the recovery from the paralysis and pain associated with polyneuritis may be extremely rapid, and thereafter it may take weeks before the patient regains the normal use of his legs. The apparent reason for this is that varying periods of time are required for the different elements in the nervous system to regain their normal function. It seems almost certain to the authors that the rapid initial recovery is due to a return of function of the nerve cells, whereas the subsequent slow progress is associated with the remyelinization of the nerve fibers. Mellanby (36) is of the opinion that the initial prompt response is due to vitamin B_1 and that the subsequent slower healing is dependent upon vitamin A. The solution of the problem is unusually difficult in the absence of knowledge concerning the effect of vitamin B_1 on cellular metabolism (see Chapter XXVI).

The mechanism of cardiovascular failure in vitamin B_1 deficiency. The beriberi heart results from failure to utilize sufficient quantities of vitamin B_1. It is characterized by two outstanding features: 1, the predominance of cardiac enlargement on the right side; 2, diminished contractile power. Lack of satisfactory anatomical or physiological explanations for these find-

ings has led to the following hypotheses as explanations of the mechanism of the heart failure.

1. *Degeneration of the vagus nerve.* Degeneration of the vagus nerve in cases of beriberi was noted many years ago and was considered the cause of cardiac failure. However, recent reports by Stanley (37), Aalsmeer and Wenckebach (38), Shimazono (39), and Keefer (11) fail to support this hypothesis. The finding of degeneration in the vagus nerve often does not correlate with the clinical manifestations of beriberi. For example, many of the acute cases with minimal changes in the vagus may have the most severe cardiac failure. Furthermore, paralysis of the vagus nerve by atropin does not produce the clinical picture of cardiac failure as seen in beriberi (40).

2. *Respiratory paralysis.* Miura (41) was the first to propose respiratory paralysis as the cause of cardiac failure in beriberi. He believed that the hypertrophy of the right side of the heart was due to retraction of the lung and to elevation of the diaphragm associated with contraction of the branches of the pulmonary arteries. There is little evidence in favor of this theory.

3. *Water retention or edema hypothesis.* This hypothesis was proposed by Aalsmeer and Wenckebach (38), who state that a deficiency of vitamin B_1 produces disturbances of metabolism which, in turn, cause the skeletal and cardiac muscles to absorb water. They believe that the edema results in loss of contractility and produces heart failure. Consistent with this theory is the observation that heart failure is much more common in the wet type of beriberi than in the dry type; moreover, pathologists can sometimes demonstrate excess fluid between and within the cardiac cells. Aalsmeer and Wenckebach have given additional support to this theory by showing that the administration of vitamin B_1 concentrate to patients with beriberi heart causes the disease "to melt away as ice in the sun." These authors base their theory on clinical observations which seem correct except for the reservation that right-sided dilatation and hypertrophy described by them and others represent only one of the cardiovascular manifestations of beriberi. Keefer (11), Weiss and Wilkins (13), and Strauss (42) have observed that some patients with beriberi do not have right-sided failure only. Why do certain patients with vitamin B_1 deficiency develop right-sided failure, left-sided failure, vasomotor collapse and shock, or some combination of these factors? Wenckebach regards the beriberi heart as being predominantly an enlargement of the right side and attempts to explain this enlargement on the following theoretical basis: In the beginning, he believes that both sides of the heart are involved equally but that the right side is certain to fail before the left in persons with beriberi. This assump-

tion he bases on the fact that the right side of the heart is less strong than the left, plus the fact that the left side cannot receive more blood than the right side is able to transmit. In other words, the more the right side of the heart fails, the less work the left side is compelled to do. The nature of the cardiovascular changes is not explained by Wenckebach. It is not clear to the authors, from Wenckebach's statement, why the right side of the heart should fail before the left in patients with beriberi. He presents little data on hemodynamics, with the exception of the response to epinephrin and pitressin. Likewise, it is the opinion of the authors and others (43) that the morphological observations concerning the edema of the heart cells are not adequate to explain the pathological physiology. In the first place, there may be no demonstrable histological changes or the changes may only be slight; and, in the second place, the changes when present are not specific, and right-sided failure may occur without necessarily being accompanied by histological changes. We do not have information which would lead us to believe that vitamin B_1 affects the cells of the right side of the heart differently, nor is there any published information to the effect that there is more edema in the heart cells of the right side than in those of the left.

Mebius (44), who accepts Wenckebach's hypothesis, states that a lack of vitamin B_1 directly affects the contractile portion of the heart muscle and thus leads directly to heart failure.

In studying patients with beriberi and closely related deficiency diseases by means of electrocardiography, Scott and Herrmann (45), Feil (12), Weiss and Wilkins (13), and others found that the conductivity was altered in certain cases. Feil, and Weiss and Wilkins pointed out that certain patients with pellagra and neuritis have tachycardia, abnormality in the T waves, and prolongation of the electrical systole (Q–T). These changes tend to disappear following dietary treatment, which suggests that they are nutritional in origin. Weiss and Wilkins have advanced evidence to show that vitamin B_1 plays a role in the development of these electrical abnormalities. Porter and Higginbotham (46) observed no electrocardiographic changes in patients with pellagra. One of the authors observed the cases studied by Feil and noted that most of the pellagrins having tachycardia had small hearts. (Experimental vitamin B_1 deficiency in animals often shows atrophy of the heart.) It is especially important to determine whether or not patients with deficiency states have cardiovascular manifestations of vitamin B_1 deficiency and whether cases similar to them occur with any degree of frequency in the Orient.

Wenckebach, and Weiss and Wilkins discuss the peripheral dilatation of the vessels. At the present time it is difficult to state what part the peripheral dilatation and increased capillary

pressure may play in leading to right-sided dilatation of the heart. In a case recently studied by Weiss (43), it seemed that the peripheral arteriolar dilatation predisposed the patient to circulatory shock and the formation of edema.

As yet we have no information as to the initial step in the mechanism of cardiovascular changes in patients with beriberi. Is it a change in the vagus tone, is it peripheral arteriolar dilatation, is it myocardial change, or is it a summation of these factors which initiates cardiovascular failure? If the peripheral vasodilatation proves to be important, is its origin peripheral or central? Certainly the cardiac involvement cannot be attributed entirely to dilatation of the peripheral vessels, as electrocardiographic changes and even dilatation of the heart can occur without appreciable dilatation of the peripheral arterioles.

Keefer has admirably summarized the present state of our knowledge of the cardiovascular changes associated with Oriental beriberi. He points out that a deficiency of vitamin B_1 causes changes in the heart characterized by cardiac enlargement and deficient contractile power. He emphasizes the point that muscular exercise plays a tremendous role in the course of the disease: if the patient is not disabled by polyneuritis and performs muscular work, cardiac insufficiency tends to result; if painful polyneuritis is present, the patient cannot perform excessive muscular work and the myocardium is protected, and hence cardiac failure does not occur. This explanation is in agreement with Smith's observation (47) that a proper diet is important in the treatment of any form of heart disease and is directly in support of Barr's recent study (48) demonstrating the importance of muscular exercise in the production of any form of heart failure. In recent studies on the role of vitamin B_1 in cardiovascular disease, Jones and Sure (49) noted a striking similarity between the symptoms of cardiac disease and accentuated cases of vitamin B_1 deficiency. They believe that it is likely that certain cardiac diseases may represent an accumulative deficiency of vitamin B_1 over a prolonged period of time.

The mechanism of edema formation in vitamin B_1 deficiency. The mechanism of edema formation in beriberi is not known. In general, we can say that it is dietary in origin, since it disappears as a result of adequate dietetic treatment and does not return so long as the diet remains adequate. Its presence indicates a perversion of the physiological mechanism which controls the interchange of fluids between the capillaries, the tissue spaces, and the lymphatic vessels (see Landis' table, Chapter I). There have been various explanations given to the origin of the edema in beriberi. In some cases the edema seems to be essentially cardiac in origin; in others it is due to an associated low plasma protein content of the blood (11, 35). There are cases of edema

reported in which the heart apparently is normal, the plasma proteins are normal, and yet the edema will disappear promptly following treatment with an adequate diet (40, 50, 51, 52). As one studies the outline of edema formation as explained by Landis (50), he can see that we have no proof in a given case of beriberi as to whether the actual factor is an elevation of capillary pressure, a lowering of colloid osmotic pressure, damage to the capillary walls, interference with lymphatic circulation, or whether it may be dependent, in part, upon a high salt intake, high fluid intake, warm environment, or disturbed innervation. Clinical observations may show that the edema in one case of beriberi may not be caused in exactly the same way as in another case. For example, in certain instances cardiac insufficiency seems to be a primary cause; in others a deficiency of colloid osmotic pressure may play a role; in still others there seems to be increased capillary pressure. Nutritional edema has been demonstrated in some instances where there is no evidence of cardiac failure and where the colloid osmotic pressure is within normal limits. In such instances the edema may disappear promptly following an adequate diet. Could this be a specific action of vitamin B_1? At the present time we do not have sufficient hemodynamic studies on typical cases of Oriental beriberi to evaluate the primary and contributory causes of edema.

Mebius (44), in 1929, accounted for all the symptomatology of beriberi on the basis of intercellular edema, and stated that the generalized edema is due entirely to a lack of vitamin B_1. He did not explain the lack of edema in the cases of dry beriberi which is known to be caused by a lack of vitamin B_1. We need an explanation as to why some cases of beriberi have edema and others do not. We can say with a relative degree of certainty that edema cannot be due entirely to a protein deficiency, since it is often cured by concentrates that contain no protein. The concentrates that cure both wet and dry beriberi may contain vitamins other than vitamin B_1. McCarrison (23) gives this explanation: that dry beriberi is caused by a complete absence of vitamin B while wet beriberi is produced by partial and long-continued deficiency of this vitamin.

Now that vitamin B_1 crystals have become available in ample quantities, we expect valuable information to be obtained by treatment of a large series of patients with wet beriberi by means of this substance. Until such information is available, no theory will be entirely satisfactory in explaining the clinical and pathological manifestations of the lack of vitamin B_1.

Vitamin B_1 deficiency and the gastro-intestinal tract. Vitamin B_1 is essential for the normal functioning of the gastro-intestinal tract. At the present time, however, it is difficult to understand how an inadequate amount of this vitamin pre-

disposes to gastro-intestinal disturbances. McCarrison (20) suggested many years ago that a deficiency of vitamin B_1 caused degeneration of Auerbach's plexus, which in turn affected the intestinal tract. An inadequate amount of vitamin B_1 has also been shown to be associated with atrophy of the glandular portions of the intestines in human beings and animals. Some investigators (17, 53, 54) are of the opinion that desire for food, normal secretion of hydrochloric acid into the stomach, and normal motility of the intestines are dependent, in part at least, upon the presence of vitamin B_1. Anorexia is the initial symptom of dietary vitamin B_1 deficiency in experimental animals and human beings. It may occur as the result of a moderate avitaminosis and in the absence of any other signs of vitamin B_1 inadequacy. Prompt disappearance of the anorexia follows the administration of vitamin B_1. As Sherman (55) states, the effect of vitamin B_1 upon the desire for food is a true one, since the vitamin B_1 deficient animal when given the vitamin "will return with appetite to the same food which it had previously refused." Cowgill and Gilman (17) relate the anorexia to hypochlorhydria and loss of muscular tone in the stomach. On the other hand, Alvarez, Pilcher, Foley, Mayer and Osterberg (56) and Sure and Harrelson (57) have been unable to demonstrate any gastric changes due to a lack of vitamin B_1.

A number of investigators (20, 21, 22) are of the opinion that ulcers of the stomach are the most definite gastro-intestinal lesions arising from vitamin B_1 deficiency. It has been stated that a lack of vitamin B_1 may produce peptic ulcer in human beings, but there is little evidence in favor of this belief (58). Investigators (20, 53, 59) have shown that animals maintained on diets deficient in vitamin B_1 have a striking increase in the size of the colon, and from this observation concluded that vitamin B_1 is necessary for the maintenance of normal muscular tone of the large intestine.

Clinical studies on a series of seventy-five patients caused Fitts (60) to conclude that "the continual slight shortage in the modern day diet of vitamin B leads to definite changes in the motor and secretory mechanism of the gastro-intestinal tract." The signs and symptoms of the avitaminosis in the seventy-five patients studied by this author led him to call attention to a syndrome of gastric atony, spastic colon, low hydrochloric acid values, and hypothyroidism. When patients having the symptoms enumerated were given vitamin B_1, a decided improvement in this condition followed.

Simpson (61) is of the opinion that eructations, flatus, headache, visceroptosis, and loss of weight are oftentimes due to gastro-intestinal changes arising from vitamin B_1 lack.

It is extremely difficult to evaluate accurately many of the

studies made on man and animals in relation to the gastro-intestinal function and the administration of yeast, wheat germ, and various other concentrates, since these substances contain many chemical constituents in addition to vitamin B_1. It is also difficult to state the exact role that a lack of vitamin B_1 may play in producing gastro-intestinal symptoms, as there is no objective method for making a diagnosis of such gastro-intestinal disorders. It seems fair to state, however, that the symptoms arising from the gastro-intestinal tract in patients or animals with clinical evidence of vitamin B_1 deficiency are likely to be caused by a lack of vitamin B_1. Controlled studies with vitamin B_1 crystals will throw light on many of these conflicting points within a short time.

Vitamin B_1 deficiency in relation to growth and maintenance of body weight. Vitamin B_1 is an essential factor for normal growth in the young and for maintenance of normal weight and health in the adult. It has been shown that an enrichment of the diet in vitamin B_1 content is followed by improvement in growth, appetite, and body weight (62, 63, 64, 65). It is essential that the diet have an adequate vitamin B_1 content when there is increased metabolism from any cause. It is the opinion of a number of clinicians that a suboptimal supply of vitamin B_1 results in limitation of growth in infancy and in childhood, and predisposes to loss of weight in the adult. It is particularly important for the physician to realize that several factors may operate at the same time to produce a deficiency, and that many therapeutic diets may be inadequate in vitamin B_1 to meet the requirements for maintenance of growth and body weight (see Chapter VIII).

Vitamin B_1 deficiency in relation to reproduction, pregnancy, and lactation. Vitamin B_1 deficiency is very often associated with loss of libido in human beings and experimental animals. Whether this is induced by general ill health or whether it is directly related to dysfunction of the sex glands has not been determined. It has been shown, however, that the gonads and pituitary may be affected by a lack of vitamin B_1 (27, 28). Tarr and McNiele (66) have demonstrated that pregnancy occurs much more often among women who have eaten large amounts of the vitamin B complex than in those whose diet is deficient in this substance. There is a great increase in the vitamin B_1 requirement during pregnancy and lactation. Hence, it is to be expected that vitamin B_1 deficiency occurs frequently during the course of pregnancy. The development and treatment of the polyneuritic symptoms have been discussed in the preceding chapter. Vomiting of pregnancy is the most common symptom. The vomiting in itself may be associated with a lack of vitamin B_1 and, in turn, may lead to further vitamin B_1 avitaminosis.

Strauss and McDonald (67) were able to relieve the vomiting and polyneuritis associated with pregnancy by means of large doses of yeast. Joachimovitz (68) reported relief of the vomiting of pregnancy following the administration of vitamin B_1 crystals. Plass and Mengert (69) recommend the use of vitamin B_1 in pregnancy as a preventive for the vomiting, polyneuritis, and mild intestinal dysfunction. Stähler (70) claims that the onset of labor in vitamin B_1 deficient individuals is delayed, that uterine contraction is weakened, and that labor in such persons can be induced by injection of vitamin B_1. Tarr and McNiele (66) state that the period of lactation is increased following the administration of vitamin B_1. It should be pointed out that vitamin B_1 crystals were not used in relation to lactation in human beings. Hence, it must be concluded that as yet we have no final evidence that vitamin B_1 plays a role in human lactation. It seems likely that mothers failing to lactate properly may predispose their babies to beriberi. A number of years ago Andrews (71) demonstrated that the milk from human mothers with beriberi induces beriberi in puppies.

Vitamin B_1 deficiency in relation to infancy and childhood. Infants are extremely susceptible to vitamin B_1 deficiency and consequently beriberi occurs more frequently in early age than later in life. Nursing mothers, though they themselves may be free from any symptoms of beriberi, often have infants who develop severe vitamin B_1 deficiency (see Chapter II). Knott (72) has found that the vitamin B_1 intake necessary for optimum growth in children is 27 International Units (about 1/10 milligram) per kilogram of body weight, or six times the minimal vitamin B_1 requirement as calculated according to Cowgill.

The relation of vitamin B_1 to carbohydrate metabolism. Peters and his collaborators (73) have recently shown that vitamin B_1 is a catalyst needed for the removal of degradation products of carbohydrate metabolism. Previously, Kinnersley and Peters (74) were able to show that lactic acid was present in the brains of pigeons with vitamin B_1 deficiency and that its presence is closely correlated with the acute manifestations of vitamin B_1 deficiency. Passmore, Peters, and Sinclair (75) have shown that in avitaminotic brain tissue, *in vitro*, pyruvate is formed from the lactate and have concluded that vitamin B_1 is concerned with the removal of pyruvate. Pyruvate is present in abnormal amounts in vitamin B_1 deficient rats and birds. Recently, Platt and Lu (76) stated that it is increased in the blood and spinal fluid of patients with beriberi. The amount of bisulfite-binding substance, to some extent, parallels the clinical manifestations of vitamin B_1 deficiency. Geiger and Rosenberg (77) have shown that there is another intermediate metabolite arising from carbohydrate that appears in infants with toxic

symptoms. They regard this substance as methyl glyoxal. According to Arakawa (78), Asakura (79), Chiba (80), and Uga (81), it occurs in large amounts in the blood of patients with beriberi and in animals with experimental beriberi. These Japanese writers consider that the methyl glyoxal is excreted as a toxin into the milk and that infants fed at the breast of mothers suffering from beriberi not only have a vitamin B_1 deficiency but also are poisoned by methyl glyoxal. Very recently Lohmann and Schuster (82) have shown that a pyrophosphate of vitamin B_1 acts as a co-carboxylase.

There are a number of other observations which show that there is a relationship between carbohydrate metabolism and vitamin B_1 deficiency. McCarrison (23) has shown that a high-carbohydrate diet intensifies the effect of a deficiency. It is known that the glucose tolerance curves are high in pigeons deficient in vitamin B_1 (83). These points are discussed in more detail in Chapter XXV, but in general, the observations on experimental animals tend to show that in vitamin B_1 deficiency there is a distinct alteration in the carbohydrate metabolism. This effect should be studied intensively in human beings.

Vorhaus, Williams, and Waterman (84) obtained improvement following the administration of vitamin B_1 crystals to two patients with diabetes mellitus. In several other clinical studies, carbohydrate tolerance has been reported as improved following yeast therapy, but some investigators have found no improvement. Von Drigalski (85) has administered vitamin B_1 to patients with diabetes mellitus without beneficial results. These discrepancies can, to some extent, be explained by the fact that it is extremely difficult to obtain controlled observations on the average diabetic patient, for it is well known that rest in bed and freedom from anxiety are often followed by disappearance of sugar from the urine. It is known that vitamin B_1 does not alter significantly the blood-sugar level in the normal person.

A hyperglycemia and glycosuria in depancreatized dogs which does not respond to insulin but is cured by thiamin plus riboflavin has been reported (86). The whole matter of vitamin B_1 deficiency in relation to sugar disturbances requires further study, as the evidence of some association of the two is strong but still obscure. (See Chapter XXV.) Before any final word can be written as to the relationship which exists between vitamin B_1 and any disease affecting carbohydrate metabolism in human beings, controlled observations on a large series of cases will be necessary.

The relation of Vitamin B_1 to hematopoiesis. In the past decade it has been shown (87, 88, 89, 90) that the vitamin B complex is effective in the treatment of certain types of anemia. It seems likely that at least a part of the benefit derived from the

administration of this complex chemical substance is due to its "tonic" effect on the gastro-intestinal tract, or more probably to the presence of Castle's extrinsic factor in the preparation of vitamin B complex which was used (91). It has been pointed out briefly in this chapter that vitamin B_1 deficiency is said to impair gastric secretion and function. In the presence of such impairment, it is possible that the iron and protein of the diet are not metabolized efficiently.

Tropical anemia and certain other macrocytic and microcytic anemias have been greatly benefited by the administration of large quantities of the vitamin B complex, and this has led to the hypothesis that the vitamin B complex probably has a specific anti-anemic action. Crystalline vitamin B_1 is one component of this complex but as yet has not been found to play a role.

Relation of vitamin B_1 to the other vitamins and to the endocrines. It is becoming increasingly clear that an improper balance between the active principles derived from the glands of internal secretion and the vitamins and other essential substances contained in food can lead to various diseases. These materials from the glands and from the foods must eventually form a suitable environment for the cells of the tissue. This mixture of tissue fluid may be altered in many ways, and if it is unsuitable, the cells suffer and disease follows. As vitamin B_1 has been available in pure form for such a short time, we do not know its precise effect on the metabolism of the various cells of the body. Its absence, however, results in cellular alteration in the nervous system, intestine, pancreas, stomach, salivary glands, liver, adrenals and gonads, and probably in other tissues.

BIBLIOGRAPHY

1. Eijkman, C.: Eine Beriberi-ähnliche Krankheit der Hühner. Virch. Arch. *148*, 523, 1897.
2. Chamberlain, W. P., Bloombergh, H. D., and Kilbourne, E. D.: A Study of the Influence of Rice Diet and of Inanition on the Production of Multiple Neuritis of Fowls and the Bearing Thereof on the Etiology of Beriberi. Philip. J. Sci. Sect. B, *6*, 177, 1911.
3. Davison, C., and Stone, L.: Lesions of the Nervous System of the Rat in Vitamin B Deficiency. Arch. Path. *23*, 207, 1937.
4. Vedder, E. B., and Chinn, A. B.: Unpublished observations.
5. Sebrell, W. H., and Elvove, E.: Observations on the Assay of the Antineuritic Vitamin. Some of the Factors Involved in the Use of the Rat Method. Pub. Health Rep. *46*, 917, 1931.
6. Vedder, E. B.: The Pathology of Beriberi. J.A.M.A. *110*, 893, 1938.

7. Vedder, E. B., and Clark, E.: A Study of Polyneuritis Gallinarum. A Fifth Contribution to the Etiology of Beriberi. Philip J. Sci. Sect. B, *7*, 423, 1912.
8. Eddy, W. H., and Dalldorf, G.: The Avitaminoses. The Williams and Wilkins Co., Baltimore, 1937.
9. Aring, C. D., Evans, J., and Spies, T. D.: Unpublished observations.
10. McCarrison, R.: Beriberi Columbarum. Indian J. Med. Research Mem. *10*, 146, 1928.
11. Keefer, C. S.: The Beriberi Heart. Arch. Int. Med. *45*, 1, 1930.
12. Feil, H.: A Clinical Study of the Electrocardiogram and of the Phases of Cardiac Systole in Pellagra. Am. Heart Jour. *11*, 173, 1936.
13. Weiss, S., and Wilkins, R. W.: The Nature of the Cardiovascular Disturbances in Nutritional Deficiency States (Beriberi). Ann. Int. Med. *11*, 104, 1937.
14. Frisch, R. A., Mendel, L. B., and Peters, J. P.: The Production of Edema and Serum Protein Deficiency in White Rats by Low Protein Diets. J. Biol. Chem. *84*, 167, 1929.
15. Karr, W. G.: Some Effects of Water-Soluble Vitamin upon Nutrition. J. Biol. Chem. *44*, 255, 1920.
16. Cowgill, G. R.: A Contribution to the Study of the Relation between Vitamin B and the Nutrition of the Dog. Am. J. Physiol. *57*, 420, 1921.
17. Cowgill, G. R., and Gilman, A. Z.: Physiology of Vitamins. XXIII. The Effect of Lack of the Vitamin B Complex on the Secretion of Gastric Juice in Dogs with Gastric Pouches. Arch. Int. Med. *53*, 58, 1934.
18. Abderhalden, quoted from Eddy and Dalldorf (8).
19. Rowlands, M. J., and Browning, E.: The Relation of Deficiency of Vitamin B to Atony of the Stomach. Lancet I, 180, 1928.
20. McCarrison, R.: Some Surgical Aspects of Faulty Nutrition. Brit. M. J. *1*, 966, 1931.
21. Dalldorf, G., and Kellogg, M.: Incidence of Gastric Ulcer in Albino Rats Fed Diets Deficient in Vitamin B (B_1). J. Exper. Med. *56*, 391, 1932.
22. Sure, B., and Thatcher, H. S.: Avitaminosis. XVI. Production of Gastric Ulcers in the Albino Rat as a Result of Specific Influence of Deficiency of Vitamin B. Arch. Path. *16*, 809, 1933.
23. McCarrison, R.: Studies in Deficiency Disease. Oxford Medical Publications, Henry Frowde and Hodder and Stoughton, London, 1921.
24. Kon, S. K., and Drummond, J. C.: The Physiological Role of Vitamin B. Part III. Study of Vitamin B Deficiency in Pigeons. Biochem. J. *21*, 632, 1927.
25. Wolbach, S. B.: The Pathologic Changes Resulting from Vitamin Deficiency. J.A.M.A. *108*, 7, 1937.

26. Ogata, T. S.: Reserat über die Reiserkrankung des Geflügels. (Kakkeähnliche Krankheit oder Polyneuritis Gallinarum.) Verhandl. d. Japanische Path. Gesellsch. *10,* 1, 1920.
27. Evans, H. M., and Bishop, K. S.: On the Relations between Fertility and Nutrition; the Ovulation Rhythm in the Rat on Inadequate Nutritional Regimes. J. Metabolic Research *1,* 335, 1922.
28. Ueno, J.: Deficiency of Vitamin B and Endocrine Glands of Female White-Rats. Jap. J. Obst. and Gynec. *17,* 267, 1934.
29. Vedder, E. B.: Beriberi and Epidemic Dropsy. Tice's Practice of Medicine, Vol. IV. W. F. Prior Co., Hagerstown, Maryland, 1936.
30. Stitt, E. R.: The Diagnostics and Treatment of Tropical Diseases, 5th Ed. P. Blakiston's Son & Co., Philadelphia, 1929.
31. Bentley, A. J. M.: Beriberi, Its Etiology, Symptoms, Treatment and Pathology. Y. J. Pentland, Edinburgh, 1893.
32. Ellis, W. G.: A Contribution to the Pathology of Beriberi. Lancet, II, 985, 1898.
33. Internal Medicine Series, Special Section on Kakke. Edited by T. Irizawa. Nanzan, Japan, 1936.
34. McLaughlin, A. J., and Andrews, V. L.: Studies on Infant Mortality. Philip. J. Sci. *5,* 149, 1910.
35. Yang, C. S., and Huang, K. K.: Beriberi in Nanking. Chinese Med. J. *48,* 20, 1934.
36. Mellanby, E.: Nutrition and Disease. Oliver and Boyd, London, 1934.
37. Stanley, A.: Beriberi and the Heart. J. Trop. Med. *4,* 351, 1901.
38. Aalsmeer, W. C., and Wenckebach, K. F.: Herz und Kreislauf bei der Beriberi-Krankheit. Wien. Arch. f. inn. Med. *16,* 193, 1929.
39. Shimazono, J.: B-Avitaminosis und Beriberi. Ergebn. d. inn. Med. u. Kinderh. *39,* 1, 1931.
40. Spies, T. D.: Unpublished observations.
41. Miura, K.: Beriberi oder Kakke. Ergebn. d. inn. Med. u. Kinderh. *4,* 280, 1909.
42. Strauss, M. B.: The Therapeutic Use of Vitamin B_1 in Polyneuritis and Cardiovascular Conditions: Clinical Indications. J.A.M.A. *110,* 953, 1938.
43. Weiss, S.: Personal communication.
44. Mebius, J.: Ödemtheorie der Beriberi und Physiologische Wirkung der Vitamin B. Archiv. f. Path. Anat. u. Physiol. u. f. Klin. Med. (Virchow's) *271,* 432, 1929.
45. Scott, L. C., and Herrmann, G. R.: Beriberi ("Maladie des Jambes") in Louisiana, with Especial Reference to Cardiac Manifestations. J.A.M.A. *90,* 2083, 1928.
46. Porter, W. B., and Higginbotham, U.: The Heart in Endemic Pellagra. South. M. J. *30,* 1, 1937.
47. Smith, F. M.: Diet and Theophylline in Treatment of Cardiac Failure. J.A.M.A. *91,* 1274, 1928.

48. Barr, D. P.: Exercise in Cardiac Disease. J.A.M.A. *91*, 1354, 1928.
49. Jones, W. A., and Sure, B.: The Role of Vitamin B_1 in Cardiovascular Diseases. J. Lab. and Clin. Med. *22*, 991, 1937.
50. Landis, E. M.: The Mechanism of Edema Formation. Modern Concepts of Cardiovascular Disease *4*, No. 11, November, 1935.
51. Elsom, K. O.: Experimental Study of Clinical Vitamin B Deficiency. J. Clin. Investigation *14*, 40, 1935.
52. Field, H.: Personal communication.
53. Sparks, M. I., and Collins, E. N.: The Role of Vitamin B_1 in Tonus of the Large Intestine. Am. J. Digest. Dis. and Nutrition *2*, 618, 1935.
54. Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1. Experimental and Clinical Observations. J.A.M.A. *105*, 1580, 1935.
55. Sherman, H. C.: Food and Health. The Macmillan Company, New York, 1934.
56. Alvarez, W. C., Pilcher, F., Foley, M. A., Mayer, A., and Osterberg, A. E.: The Effects on the Gastric Juice of Man of Six Weeks' Deprivation of Vitamin B_1. Am. J. Digest. Dis. and Nutrition *3*, 102, 1936.
57. Sure, B., and Harrelson, R. T.: Enzymic Efficiency in Avitaminosis: Peptic Digestion in Vitamin B Deficiency. Am. J. Digest. Dis. and Nutrition *4*, 177, 1937.
58. Hartung, E. F.: Role of Vitamins and Infection in Peptic Ulcer. New York State J. Med. *32*, 914, 1932.
59. Cowgill, G. R., Deuel, H. J. Jr., Plummer, N., and Messer, F. C.: Studies in the Physiology of Vitamins. IV. Vitamin B in Relation to Gastric Motility. Am. J. Physiol. *77*, 389, 1926.
60. Fitts, J. B.: Motor and Secretary Dysfunction of the Gastro-intestinal Tract and the Vitamin B_1 Factor: A Clinical Study. South. M. J. *28*, 920, 1935.
61. Simpson, I. A.: Recent Work on Vitamins and Hormones. Malayan M. J. *11*, 1, 1936.
62. Morgan, A. F., and Barry, M. M.: Underweight Children: Increased Growth Secured through the Use of Wheat Germ. Am. J. Dis. Child. *39*, 935, 1930.
63. Dennett, R. H.: Routine Use of the Vitamin B Factor in Infant Feeding. J.A.M.A. *92*, 769, 1929.
64. Ross, J. R., and Summerfeldt, P.: Value of Increased Supply of Vitamin B_1 and Iron in the Diet of Children. Am. J. Dis. Child. *49*, 1185, 1935.
65. Tisdall, F.: Inadequacy of Present Dietary Standards. Can. Med. Assn. J. *33*, 624, 1935.
66. Tarr, E. M., and McNiele, O.: Relation of Vitamin B Deficiency to Metabolic Disturbances during Pregnancy and Lactation. Am. J. Obst. and Gynec. *29*, 811, 1935.

67. Strauss, M. B., and McDonald, W. J.: Polyneuritis of Pregnancy, a Dietary Disorder. J.A.M.A. *100,* 1320, 1933.
68. Joachimovitz, P.: Oester. Arzt. *12,* 338, 1936. (Quoted from the Story of Vitamin B_1. Merck and Company, Inc., Rahway, N. J.)
69. Plass, E. D., and Mengert, W. F.: Gestational Polyneuritis. J.A.M.A. *101,* 2020, 1933.
70. Stähler, F.: B_1-Hypovitaminosen in der Schwangerschaft. Münch. med. Wochschr. *84,* 327, 1937.
71. Andrews, V. L.: Infantile Beriberi. Philip. J. Sci. Sect. B, *7,* 67, 1912.
72. Knott, E. M.: A Quantitative Study of the Utilization and Retention of Vitamin B by Young Children. J. Nutrition *12,* 597, 1936.
73. Peters, R. A.: The Biochemical Lesion in Vitamin B_1 Deficiency. Application of Modern Biochemical Analysis in Its Diagnosis. Lancet, I, 1161, 1936.
74. Kinnersley, H. W., and Peters, R. A.: Observations upon Carbohydrate Metabolism in Birds. I. The Relation between the Lactic Acid Content of the Brain and the Symptoms of Opisthotonus in Rice-Fed Pigeons. Biochem. J. *23,* 1126, 1929.
75. Passmore, R., Peters, R. A., and Sinclair, H. M.: On Catatorulin. A New Method of Comparing the Oxidative Factor in Vitamin B_1 Concentrates. Biochem. J. *27,* 842, 1933.
76. Platt, B. S., and Lu, G. D.: Chemical and Clinical Findings in Beriberi with Special Reference to Vitamin B_1 Deficiency. Quart. J. Med. *5,* 355, 1936.
77. Geiger, A., and Rosenberg, A.: Methylglyoxal im Harne und in der Cerebrospinalflüssigkeit bei Ernährungsstörungen der Säulinge mit Toxischen Symptomen und bei der Experimentellen B_1-Avitaminose bei Hunden und Ratten. (Vorläufige Mitteilung.) Klin. Wochschr. *12,* 1258, 1933.
78. Arakawa, T.: An Exceedingly Sensitive Peroxidase Reagent for Human Milk. Tohoku J. Exper. Med. *16,* 83, 1930.
79. Asakura, K.: Studies on the Detoxicating Hormone of Liver (Yakriton). Effect of Yakriton on Negative Arakawa's Reaction. Tohoku J. Exper. Med. *19,* 269, 1932.
80. Chiba, M.: Prolongation of Short-timed Peroxidase Reaction of Leucocytes as a Sign of Avitaminosis B and the Effect of Vitamin B on It. A Clinical Experiment. Tohoku J. Exper. Med. *19,* 486, 1932.
81. Uga, Y.: Arakawa Reaction and Calcium Content of Human Milk. Tohoku J. Exper. Med. *25,* 169, 1935.
82. Lohmann, K., and Schuster, Ph.: Über die Co-Carboxylase. Naturwissenschaften *25,* 26, 1937.
83. Lepkovsky, S., Wood, C., and Evans, H. M.: Glucose Tolerance in Avitaminosis Due to Low Antineuritic Vitamin B. J. Biol. Chem. *87,* 239, 1930.

84. Vorhaus, M. G., Williams, R. R., and Waterman, R. E.: Studies on Crystalline Vitamin B_1: Observations in Diabetes. Am. J. Digest. Dis. and Nutrition *2*, 541, 1935.
85. von Drigalski, V. W.: Vitamin B as a Substitute for Insulin. Arch. f. Verdauung. Krankh. *57*, 1, 1935.
86. Martin, R. W.: Vitaminfreie Ernährung und Insulinwirksamkeit. Zeitschr. physiol. Chem. *248*, 242, 1937.
87. Vaughan, J. M., and Hunter, D.: The Treatment by Marmite of Megalocytic Hyperchromic Anaemia Occurring in Idiopathic Steatorrhea (Coeliac Disease). Lancet I, 829, 1932.
88. Goodall, A.: The Treatment of Pernicious Anemia by Marmite. Lancet II, 781, 1932.
89. Witts, L. J., and Fairley, N. H.: Discussion on the Megalocytic Anemias of Tropical and Non-Tropical Countries. Proc. Roy. Soc. Med. *25*, 1703, 1932.
90. Ungley, C. C., and James, G. V.: The Effect of Yeast and Wheat Embryo in Anaemias: II. The Nature of the Haemopoietic Factor in Yeast Effective in Pernicious Anemia. Quart. J. Med. *3*, 523, 1934.
91. Strauss, M. B., and Castle, W. B.: The Nature of the Extrinsic Factor of the Deficiency State in Pernicious Anemia and in Related Macrocytic Anemias. Activation of Yeast Derivatives with Normal Human Gastric Juice. New Eng. J. Med. *207*, 55, 1932.

CHAPTER V

PHARMACOLOGY OF VITAMIN B_1

At the present time the pharmacological action of vitamin B_1 is not well understood, but studies along this line are being stimulated by the availability of the pure vitamin in large amounts.

The role of vitamin B_1 in carbohydrate metabolism and its possible identity with a coenzyme in the oxidation-reduction system of the animal organism are discussed in Chapter XXVI.

Hecht and Weese (1) reported that subcutaneous injections of from 50 to 500 mg./kg. of body weight resulted in a 20 per cent increase in the oxygen consumption of guinea pigs within the first half hour; this was accompanied by a decrease in the R–Q from 0.80 to 0.75. The basal metabolic rate returned to normal in the course of a few hours. The oxygen consumption of a group of vitamin B_1 depleted rats observed by Molitor and Sampson (2), all showing neuro-muscular disturbances, was markedly increased only after the animals had received food.

That vitamin B_1 has a protective action against the toxic effects of thyroxin has been reported by Sure and Buchanan (3), who found that the pure crystalline vitamin in doses of 30 to 100 micrograms counteracted the "toxicity" of as much as 0.2 milligram of thyroxin daily in animals receiving a diet containing autoclaved brewers' yeast. For a more extended abstract of this work the reader should refer to Chapter XX.

In regard to the level of the blood sugar, Hecht and Weese (1) state that with subcutaneous injections of 0.1 gm./kg., the fasting blood sugar of rabbits remained within the normal range for eight hours. A toxic dose, 0.5 gm./kg., however, produced a slight increase in the level, which returned to normal within six hours. The level of the blood sugar in normal human beings was not altered, and the vitamin did not affect the normal action of insulin.

According to Tislowitz (4), who used dogs as experimental animals, capillary permeability is decreased by vitamin B_1, as well as by vitamin C, so that Congo red escapes slowly from the blood stream. Tislowitz also indicated that the vitamin seems to exert an inhibitory action on inflammatory processes.

Vitamin B_1 apparently possesses diuretic properties. Hecht

and Weese (1) were able to double the excretion of urine in fasting rabbits, after the oral administration of 100 grams of water, by the injection of 0.1 gm./kg. of the vitamin. Molitor and Sampson (2) have shown that water diuresis of a group of rats decreases some 30 per cent during the depletion period and that the diuresis returns to normal in about fifteen hours after the administration of 50 micrograms of vitamin B_1 hydrochloride to each rat. Leong (5) and Helmer (6), among others, have demonstrated that an excess of vitamin B_1, administered to animals and to human beings, is promptly excreted in the urine.

Vasodilatation, according to Molitor and Sampson (2), occasionally followed the intravenous injection of very large sublethal doses of vitamin B_1 into unnarcotized rabbits. Hecht and Weese (1) found that rapid injection of 30 mg./kg. caused a maximum fall in the systolic blood pressure of 35 mm. Hg in narcotized cats. The influence on the blood pressure in narcotized rabbits was observed to be similar, except that the fall was not so acute. The first lowering occurred with 10 mg./kg., and even after 80 mg./kg. had been injected, the fall was only 18 mm. In decapitated novocainized cats, 100 mg./kg. given intravenously had no effect. The observed blood pressure fall is believed by these authors to be due to a central injury.

The effect of vitamin B_1 on the heart of the normal dog has been studied by Tislowitz and Pines (7) by means of the electrocardiograph. One hour after a single injection of 10 milligrams to dogs narcotized with chloral and curare, bradycardia with marked respiratory arrhythmia and sudden death occurred. This effect, they believe, is produced by vagotonic action of central origin and is related, perhaps, to changes in the carbohydrate metabolism of the deep centers of the brain. In the opinion of the authors, the evidence is not of such nature as to warrant this conclusion. Administration of smaller doses, from 1 to 2 milligrams, produced in unnarcotized dogs similar effects of bradycardia and sinus arrhythmia, which, however, soon disappeared after the dosage was discontinued. Tislowitz and Pines also report that injections of adrenalin superimposed upon vitamin B_1 vagotonia gave rise to a typical paroxysmal tachycardia of supraventricular origin. Molitor and Sampson (2) found no change in the heart rate of normal rats following intravenous injections of 10 mg./kg. of vitamin B_1, or in rats whose heart rate had been previously decreased by the administration of bulbocapnin phosphate (80 mg./kg.). It is well known that bradycardia develops during vitamin B_1 depletion; the heart rate may decrease as much as 50 per cent. In vitamin B_1 deficient animals, the injection of 10 mg. of vitamin B_1 was followed by a 30 per cent increase in the heart rate within one and one-half hours. Within four days the heart rate had returned to normal.

Hecht and Weese (1) have reported that isolated frog heart, as well as the isolated small intestine of the rabbit and guinea pig, showed no reaction in concentrations of vitamin B_1 up to 1:1000. Moreover, Molitor and Sampson (2) have shown that vitamin B_1 produced no effect on the motility of the rabbit's intestine.

The rate of respiration is generally decreased by excessive doses of vitamin B_1. As observed in narcotized cats by Hecht and Weese (1), the respiration was practically uninfluenced by injected doses of vitamin B_1 up to 20 mg./kg. Higher doses effected the usual change.

Wilder (8) and Aring, Evans, and Spies (9) have used large amounts of vitamin B_1 in the treatment of epilepsy in human beings. The results in both series were negative. Molitor and Sampson (2) have shown that vitamin B_1 has no effect on thujone convulsions in the rat, provided the animal has a diet adequate in vitamin B_1. The animals depleted of this vitamin have a marked increase in the number of convulsions, which returns to normal four days after the administration of 20 micrograms of vitamin B_1 hydrochloride. But a restricted diet produces similar results, even if an excess of vitamin B_1 is present. The intravenous injection of 10 mg./kg. failed to effect any change in the type and duration of the nystagmus in normal rats.

Perla (10) reports that in rats receiving large doses of synthetic vitamin B_1, there is interference with lactation and normal nursing in the second generation. These observations should be checked carefully, as his findings do not coincide with those of other investigators. Not infrequently, cannibalism occurs in the first litters, particularly if the young are handled. It would add information of great importance if the females were bred repeatedly. At any rate, the doses administered were far beyond the therapeutic range, and these observations should in no way deter the physician from administering vitamin B_1 whenever it is indicated in therapy.

For further information on the relation of vitamin B_1 to appetite, growth, and lactation, the reader should refer to the preceding chapter.

Toxicology

The lethal doses of vitamin B_1 in experimental animals, as established by Molitor and Sampson (2), are as follows: injected intravenously, for mice 125 mg./kg. of body weight; rats, 250 mg./kg.; rabbits, 300 mg./kg; dogs, 350 mg./kg. Hecht and Weese (1) have supplied data approximating these results, particularly for mice and rabbits. The ratio for the toxic dose when vitamin B_1 is administered intravenously, subcutaneously, and orally has been shown by the former pair of investigators to be

1:6:40. Molitor and Sampson observed that repeated subcutaneous or intramuscular injections were accompanied by subjective or objective signs of irritation in the mouse, cat, and guinea pig. These symptoms did not occur in the rabbit, dog, or monkey. Symptoms preceding death as observed for all species studied were: weakness, general muscular tetany, occasional cramplike spasm, labored breathing which became slow and irregular. Death resulted from respiratory collapse. The heart continued beating from one to two minutes after the respiratory failure, and the blood pressure generally fell only after breathing ceased completely. The death rate was the same in narcotized and unnarcotized animals. In the experiments of Hecht and Weese on two rhesus monkeys, the first toxic symptoms were observed with injection of 600 mg./kg. A single therapeutic dose is generally from 1 to 5 mg. Thus the ratio of the effective therapeutic dose to the minimal toxic dose is between 1:7,200 and 1:36,000—"a condition which has not been equalled in any other synthetic or natural therapeutic agent."

The possibility of a cumulative toxicity from repeated administration of large doses of vitamin B_1 has been investigated by Hecht and Weese. Rabbits receiving intravenously 50 mg./kg. daily for two weeks manifested no acute effects other than occasional minor symptoms suggestive of collapse, which occurred immediately after injection and which were overcome within a few minutes. During the course of the experiment there was no loss of body weight or appetite. The daily urine showed no pathological changes, the hemoglobin content and erythrocyte count remained normal, and the blood pressure and electrocardiograph remained unchanged. Histological examinations of the tissues at the end of the experiment showed no pathological changes. The results of experiments on the rhesus monkeys were also negative in regard to possible cumulative toxicity of this vitamin.

Because of the great difference between the therapeutic and toxic dose, and the absence of evidence of cumulative toxicity, vitamin B_1 is a remarkably safe therapeutic agent and may be used without concern in long-extended treatment. In man the therapeutic dosage is, at most, relatively few milligrams per day. The authors have given to normal people 500 milligrams daily for a month without any objective toxic effects. Intravenous injections of ten, fifteen, or even twenty times the normal dose have caused no noticeable symptoms, except that patients receiving large amounts of crystalline vitamin B_1 experience a yeastlike taste.

BIBLIOGRAPHY

1. Hecht, G., and Weese, H.: Pharmakologisches über Vitamin B_1 (Betaxin). Klin. Wochschr. *16,* 414, 1937.
2. Molitor, H., and Sampson, W. L.: On the Action of Large Doses of Natural and Synthetic Vitamin B_1. E. Merck's Jahresbericht, p. 51, 1936.
3. Sure, B., and Buchanan, K. S.: Antithyrogenic Action of Crystalline Vitamin B. J. Nutrition *13,* 513, 1937.
4. Tislowitz, R.: Wirkung der Vitamine B_1 und C auf die Dauer des Verbleibens von Kongorot in der Blutbahn. Biochem. Ztschr. 291, 70, 1937.
5. Leong, P. C.: Vitamin B_1 in the Animal Organism: I. The Maximum Storage of Vitamin B_1 in Tissues of the Rat. Biochem. J. *31,* 367, 1937.
6. Helmer, O. M.: Determination of Vitamins B and G in Human Urine by Rat-growth Method. J. Nutrition *13,* 279, 1937.
7. Tislowitz, R., and Pines, I.: Über die Vagotonische Wirkung des Vitamins B_1 auf das Normale Hundeherz. Zur Wirkung des Vitamins auf das Zentralnervensystem. Klin. Wochschr. *16,* 923, 1937.
8. Wilder, R.: Personal communication.
9. Aring, C., Evans, J., and Spies, T. D.: Unpublished observations.
10. Perla, D.: Toxic Effects of an Excess of Vitamin B_1 in Rats. Proc. Soc. Exper. Biol. and Med. *37,* 169, 1937.

CHAPTER VI

PREVENTION AND TREATMENT

Prevention

The physician and health officer have been defending the community against infectious diseases for many years. In view of the recent advances in nutrition, the community should soon be better protected against dietary deficiency diseases than it is at present. Such deficiency diseases can be eradicated only when adequate amounts of a well-balanced diet are available to and assimilated by all. During the past few years science has made possible immense improvements in the standards of living, but complete achievement of the ideal cannot be realized until the food supply of the people as a whole has been improved and the masses informed as to the meaning of optimal nutrition. Thus, poverty and ignorance are two principal problems which must be solved if vitamin B_1 deficiency is to become a disease of the past. Poverty is intimately associated with the high incidence of beriberi in the Orient, and our greatest hope of prevention lies in increased prosperity for all. In addition to poverty, a lack of information as to the need for an adequate and well-balanced diet and for special vitamin B_1 supplements is responsible for the high incidence of vitamin B_1 deficiency. It is a common fallacy to believe that one's customary diet is adequate or that appetite is a correct guide to the proper selection of food. Education of the people is imperative so that those who can afford to buy the relatively inexpensive protective foods will do so, whereas those who cannot afford them will obtain supplements such as dried yeast, wheat germ, or rice polishings through the proper relief agencies. Prevention in the general population might be obtained if manufacturers incorporated vitamin B_1 in cereals and other refined foods.

The Health Committee of the League of Nations has made the initial move toward the objective of universal optimal nutrition by establishing figures for man's requirements for the various dietary factors necessary for normal physiological function (1).

Until such time the incidence of beriberi and subclinical forms of vitamin B_1 deficiency can be greatly decreased by the application of the following recommendations:

1. Fresh foods such as potatoes, native vegetables, pork and liver, eggs, milk and fruits, and especially beans and whole grain cereals should be substituted for or added to a diet of decorticated cereals and dried or canned foods whenever possible.

2. The use of dried yeast, wheat germ, liver, kidney, and vitamin B_1 crystals as supplements to the diet should be encouraged whenever possible. The expense of such materials, however, often interferes with their extensive use.

3. Since vitamin B_1 is water-soluble, a large amount of it is lost when the water in which the foods are cooked is thrown away. It is recommended, therefore, that the water in which foods containing vitamin B_1 are cooked be used for broths. Whole barley or other grains which are rich in vitamin B_1 may be added to such broths to insure additional protection.

4. The use of undermilled rather than overmilled grains should be rigidly enforced among government troops and in prisons, asylums, and other institutions where mass feeding is required (see Chapter VII). Each lot of grain purchased should be examined to determine whether there is an adequate amount of the germ and of the external layers of the grain. Since molds, weevils, and other infestations deprive cereals of this vitamin, one must be certain that the grain has not spoiled.

5. Particular attention should be directed toward the diets of persons with chronic debilitating diseases and increased metabolism, for the incidence of vitamin B_1 deficiency is high among such people. Chronic alcoholic addiction, pellagra, sprue, pernicious anemia, colitis, diabetes mellitus, tuberculosis, senility, malignancy, cirrhosis, and other diseases may interfere with the proper nutrition of the patient and thus predispose to the development of vitamin B_1 deficiency.

6. The diets of pregnant and lactating women should be especially rich in vitamin B_1. Whenever there is any doubt as to the adequacy or utilization of this vitamin either in the mother or in the child, supplements should be given. The supplements should be continued in the mother and child until the proper diet is assured. The nursing mother should receive at least 5 milligrams of thiamin or its equivalent daily; the infant should receive 0.5 milligram per day or its equivalent, and children should receive 1 milligram or its equivalent per day.

7. The requirement for vitamin B_1 may be distinctly above the average in persons with fever, severe gastro-intestinal symptoms, hyperthyroidism, and other conditions. It is essential for the physician to prescribe amounts above the average for such persons. The authors suggest for such a maintenance dose, from 5 to 10 milligrams of pure vitamin B_1 or its equivalent, except when the patient is unable to absorb from the gastro-intestinal tract. In such instances it is essential that vitamin B_1 be given

parenterally in order to protect the person from a deficiency of this vitamin.

Treatment

Clinical indications for vitamin B_1 therapy. Now that pure vitamin B_1 is available for clinical use, there is a tendency for the physician to administer it in many diseases. Undoubtedly many patients who are now being given vitamin B_1 do not have a vitamin B_1 lack and therefore are not benefited by it. Others who could be benefited by this substance are not given it, or if the vitamin is prescribed, it is prescribed in too small amounts. The present unsatisfactory state of knowledge is due to lack of precise study. Of course, it is axiomatic that an adequate and well-balanced diet is of utmost importance for health. When the nature of the patient's disease causes the doctor to reduce certain kinds of foods in the dietary, it is essential that supplements be prescribed in such a way that all necessary elements are supplied in the required amounts. But controlled studies are necessary before we have final knowledge as to the precise indications for vitamin B_1 therapy, and the minimal and optimal requirements for man.

The Council of Pharmacy of the American Medical Association (2) has recently stated that it considers the following to be allowable claims for vitamin B_1 function:

1. May be cited as of value in correcting and preventing absence or loss of appetite—anorexia—of dietary origin in certain cases.
2. Of value in securing optimal growth of infants and children.
3. Of value in correcting and preventing beriberi.
4. Because it is a dietary essential its administration in concentrated form is of value in some conditions where difficulty in utilizing ordinary foods in the usual way is encountered.
5. There are many experimental indications in the literature indicating other possible functions of vitamin B, *e.g.*, an influence on intestinal motility and neuritis of various types and also indications of greatly augmented requirement when metabolism has increased as in hyperthyroidism, neuritis of various types, and infection. It seems too early to permit advertising claims for these items.

The authors support these claims and the method in which they are presented. However, we feel that since the time of publication, in 1936, additional information has become available which indicates that persons having polyneuritis associated with chronic alcoholism, pellagra, or pregnancy are greatly benefited by the administration of this material. There is enough clinical information available at the present time to warrant the trial of thiamin in patients who have peripheral neuritis sec-

ondary to dietary deficiency or associated with undernutrition from any cause. Also a trial is warranted in peripheral neuritis and edema of undetermined origin, though it is obviously too early to permit advertising claims for this latter group.

Vitamin B_1 has been recommended in the treatment of diseases of the eye, ear, nose, and throat (3, 4, 5, 6), for treatment of arthritis (7, 8), as part of the insulin shock therapy of schizophrenia (9), for pain following gunshot wound in the head (10), and for many other conditions (11, 12). The authors are unable to evaluate such recommendations, but it is our belief that vitamin B_1 is useful in the treatment of such disorders only if they are caused by a deficiency of vitamin B_1. It is often difficult to make a clinical diagnosis of vitamin B_1 deficiency with any degree of assurance, and certainly many subclinical cases go unrecognized. This leads to trial and error methods of diagnosis and therapy.

It is hoped that in the near future, simple diagnostic clinical and laboratory methods will become available. Very recently, Meiklejohn (13) has applied a mold-growth test for determining the vitamin B_1 content of the blood (see page 220). Using a fluorescence method, Cline and his associates (14) have devised a method for determining excretion of vitamin B_1 in the urine (see page 217). These tests may be a distinct aid in determining whether vitamin B_1 is indicated for such miscellaneous groups of diseases. Until vitamin B_1 deficiency can be diagnosed with certainty, the physician should prescribe this substance whenever the clinical indications warrant its use. The dosages of thiamin recommended later in this chapter have been shown to be harmless and yet effective. A steady increase in its use will lead to quantity production and to a resulting decrease in cost. Yet its widespread, unwarranted use will lead to disappointment and will likely cause this form of therapy to fall into disrepute.

General principles of treatment. The more accurate the initial diagnosis of vitamin B_1 deficiency, the more likely is the physician to avoid futile treatment with vitamin B_1 preparations. In the treatment of vitamin B_1 deficiency, the problem is simply one of administering adequate amounts of the deficient substance in a way in which it can be utilized. In the adult or infant the physician should direct therapy along three lines: 1. There should be elimination of conditions causing excessive requirement for vitamin B_1 whenever possible. 2. There should be administration of the substance in adequate amounts to correct the deficiency. 3. There should be symptomatic treatment and treatment for coexisting diseases.

Irrespective of predisposing conditions and coexisting diseases, the authors consider the manifestations of vitamin B_1 deficiency as beriberi. It is axiomatic that vitamin B_1 therapy is

always indicated when there is an inadequacy of vitamin B_1, but it is often difficult and sometimes impossible to demonstrate clinically whether a vitamin B_1 deficiency exists. Hence the absolute indications for treatment depend upon the "clinical information" available to the physician.

Method of treatment. Since the minimal, maximal, and optimal doses of none of the therapeutic substances are known and since, in the experience of the authors, the following amounts have been effective, they are tentatively recommended. No routine of treatment can be satisfactorily followed. Each patient should be considered individually and the best method employed in each case. Each patient ill enough to be hospitalized should be considered as an emergency. Any change for the worse or any failure to take and retain either the food or therapeutic agents should be a signal for redoubled efforts. In such instances careful and frequent attention by the physician, nurse, and dietitian is desirable to meet varying conditions and to see that the necessary regime is carried out. Any patient severely ill with vitamin B_1 deficiency should remain in bed until his condition warrants greater freedom. Sedatives and adequate nursing care often make this less irksome. On a number of occasions the authors have noted that when, against advice, a very ill patient got up to go to the toilet, he was worse within a short period of time. In such severe cases special nursing or special attention by general duty nurses or members of the family is necessary to insure complete rest in bed and co-operation in the other essential features of treatment. In order to treat a patient properly, it is often necessary to combine specific therapy, symptomatic therapy, and treatment of co-existing diseases. For ease of description, however, they will be discussed separately.

Specific treatment in the adult and infant. The essence of treatment lies in the administration of adequate amounts of foods rich in vitamin B_1, supplemented, if possible, with large amounts of a specific therapeutic agent. The foods included in the dietary will depend upon the age, race, habits, tastes, and financial status of the patient concerned.

Adult. A well-rounded diet of 4500 calories, including fresh native vegetables and fruits, whole-grain cereals, peas, beans, barley, pork and liver, eggs, milk, and other foods of high vitamin B_1 value, should be the objective. Whenever possible, this diet should be supplemented with one or several of the following curative therapeutic substances, for even in chronic resistant cases which are cured slowly, these substances are of benefit in relieving symptoms and in shortening convalescence: dried brewers' yeast (6 ounces daily); wheat germ (6 ounces daily); extract of rice polishings (tikitiki (15) (3 ounces daily); or crystalline vitamin B_1 (10 milligrams twice daily). In cases

of severe beriberi even larger doses of crystalline vitamin B_1 (thiamin) may be indicated. In such cases it seems wise to administer 20 milligrams twice per day until the signs of avitaminosis have disappeared. In cases of mild deficiency, doses of 5 milligrams per day are adequate.

Infant. Infantile beriberi is treated best by giving intramuscularly or intravenously 5 to 10 milligrams of thiamin in sterile physiologic solution of sodium chloride, twice per day. As in the adult, the action is more prompt and more efficacious when the substance is given parenterally than when given by mouth. When injections cannot be given conveniently, or when the infant is convalescent, 10 milligrams of crystalline vitamin B_1 or an alcoholic extract of 100 grams of rice polishings (tikitiki extract) may be given orally each day. Tikitiki can be administered easily with a medicine dropper. Obviously, satisfactory anti-beriberic treatment of the mother will aid greatly in the treatment of the nursing child.

Parenteral injections are always indicated, irrespective of age or any other condition, when vitamin B_1 deficiency is associated with severe cardiac failure, severe peripheral neuritis, or severe gastro-intestinal disturbances. When the patient, infant or adult, is extremely ill, we recommend that at least the initial treatment be parenteral. There can be no question that the oral administration of thiamin in adequate doses proves efficacious in the average case of vitamin B_1 deficiency; however, it must be recognized that patients are encountered who are refractive to oral medication. In view of this fact, solutions of thiamin for parenteral injection may be required. The advantages of parenteral therapy over oral treatment include: 1. Parenteral administration causes more rapid improvement in the patient. 2. There is the certainty that the patient will actually absorb it. A deranged gastro-intestinal tract cannot serve as a block to the absorption of the medication. 3. Massive doses of the active substance can be given more easily. 4. The management of the patient is so arranged that the medication is given under the supervision of the physician, thus eliminating carelessness or other possible interferences.

Symptomatic treatment in the adult and infant. Other than vitamin B_1, there are no specific drugs of value in the treatment of beriberi. However, symptomatic treatment often affords the patient some relief. All patients with beriberi should rest as much as possible. Complete rest is especially indicated in cases with cardiac involvement, and venesection is sometimes indicated in patients who have acute congestive failure. Extensive pleural effusions should be relieved by tapping. When the peripheral nerves are involved, protection of the paralyzed muscles with splints and cradles is essential. Massage of the muscles is of

prime importance in cases having severe muscular atrophy, and graduated exercises are of great value during convalescence.

Treatment of co-existent diseases in adult and infant. Some of the diseases which predispose to beriberi are discussed in Chapters I, II, and III. These can be conveniently classified into three groups: 1. Diseases such as hyperthyroidism and fevers which are associated with an increase in general metabolism. 2. Diseases which require special diets which may be deficient in vitamin B_1. Liquid diets, soft solid diets, high carbohydrate diets, reducing diets, and diets for diabetes, heart disease, peptic ulcer, and many other diseases are often deficient in this vitamin. 3. Diseases accompanied by dietary idiosyncrasies or digestive disturbances which cause improper ingestion, assimilation, or utilization of food. Parenteral administration of vitamin B_1 is often necessary in order to provide adequate amounts of the vitamin for patients in this group. The appropriate treatment of these diseases often aids indirectly in effecting relief from the symptoms of beriberi itself. In general, these diseases, like classical beriberi, should be treated by supplying optimum nutrition. At times it is necessary for the physician to modify the method of treatment owing to some complication. However, specific therapy, whenever indicated for any of the other diseases, should be given.

The *clinical response* to vitamin B_1 therapy is often dramatic. In the acute cases, beginning improvement may be noticed within a few hours; the mild cases are often relieved within twenty-four to forty-eight hours. In the chronic case there is often great relief, particularly from pain, within a few days, though spasticity and alteration of physical signs may remain for a long period. Occasionally, in chronic cases, some residual changes remain indefinitely. It is generally recognized that the maintenance requirement and the curative dose vary from one individual to another and may vary in the same individual from time to time. These variabilities necessitate close medical supervision at all times.

Available Preparations of Vitamin B_1

Samples of several hundred available preparations containing vitamin B_1 have been obtained by the authors, and, whenever possible, the advertising material has been examined. The conclusion which we were forced to draw is that the claims made by some of the manufacturers are unwarranted because such claims have not yet been substantiated by laboratory or clinical investigation. In some instances the preparation actually contains little vitamin B_1, in others the potency varies, and in many the potency has not been determined. One cannot impress the

physician too strongly with these facts in order that he may prescribe preparations of known potency which are manufactured by a reputable concern. In this way only can the physician be certain that the patient with vitamin B_1 deficiency will have a chance to be benefited. Even among those concerns trying to determine the exact potency and to make just claims as to the therapeutic value of the preparation, difficulties arise because of variations in determining and expressing vitamin B_1 units (see Chapter XVII, pages 213–6). A great deal of the early work was reported in terms of Sherman Units. The Sherman method of assay depends upon the growth response of the rat to graded doses of the vitamin B_1 preparations which are being tested. In different laboratories, variable results are obtained on the same preparation because of the great variety of methods used, the variation in the growth rates of different strains of animals, and insufficient knowledge of the effects of other B factors. Therefore it seemed more satisfactory to determine the vitamin B_1 potency in terms of a standard substance. However, the International Standard material chosen for this purpose has proved to provide the vitamin in a form which is only partly available to experimental animals in an advanced state of deficiency, and further confusion has resulted (see Chapter XVII). It seems unwise that an adsorbate or other crude form should still be used as a standard when pure thiamin hydrochloride is now available. Great difficulty arises when attempts are made to convert Sherman Units into International Units or to convert Sherman or International Units into gamma of thiamin hydrochloride. In some laboratories one International Unit is considered to be equivalent to 1.3 Sherman Units, while in other laboratories one International Unit is said to be equivalent to 4 Sherman Units. The usual value is that one International Unit is equivalent to approximately 2 Sherman Units. Apparently, some of the commercial concerns wish to continue the use of Sherman Units simply because the number of units is greater when expressed in these units than when expressed in terms of International Units. Many commercial concerns do not describe any assay; they simply make claims. All of these problems add greatly to the confusion and make it impossible to assess fair value. It would seem distinctly worth while for some laboratory to obtain a number of preparations and to determine accurately the relative vitamin B_1 content of each, against the standard of pure vitamin B_1 crystals. In that way, and in that way alone, would one have comparative data of value. For example, some of the yeast preparations may be grown under extraordinarily constant conditions and as a result will have a relatively constant value. Such products are standardized, even though the number of vitamin B_1 units may not have been declared. There are, how-

ever, other brands of yeast which are in the nature of a by-product, and these brands are usually not grown under standard conditions. The authors have had personal experience with various commercial preparations of wheat germ, yeast, liver, ventriculin, vitamin B concentrates, and pure thiamin, both in the laboratory and in the clinic. Any of these preparations, when potent, may be of great use to the person with a vitamin B_1 deficiency, but obviously, none of these preparations can replace entirely a well-balanced diet. Our experience shows that some of the preparations are much less potent than others, and we are compelled to conclude that it is a risk for the patient to be treated with preparations of unknown therapeutic value. Synthetic vitamin B_1, which has been shown to be as effective therapeutically as the natural vitamin, is of unquestioned potency and is dispensed by a number of commercial concerns.

BIBLIOGRAPHY

1. Boudreau, F. G.: The International Campaign for Better Nutrition. The Milbank Memorial Fund Quarterly, New York, *15*, 103, 1937.
2. The Status of Certain Questions Concerning Vitamins. Report of Council on Pharmacy and Chemistry. J.A.M.A. *106*, 1732, 1936.
3. Cody, C. C.: Relation of Vitamins A, D, B and G to Otolaryngology. Arch. Otolaryng. *16*, 661, 1932.
4. Benedict, W. L., and Wagener, H. P.: Nutritional Diseases and the Eye. The Role of Vitamin B. Am. J. Med. Sci. *192*, 296, 1936.
5. Selfridge, G.: Can Certain Diseases of the Ear, Nose and Throat, Especially Degeneration of the Eighth Nerve, Be Classified as "Deficiency Diseases"? Laryngoscope *46*, 85, 1936.
6. Shastid, T. H.: Optic Neuritis and Vitamin B. Am. J. Ophth. *12*, 903, 1929.
7. Hall, F. C., and Myers, W. K.: Diet in Chronic Arthritis. Arch. Int. Med. *55*, 403, 1935.
8. Steinberg, C. L.: Vitamin B Complex Therapy in Chronic Arthritis. Am. J. Digest. Dis. and Nutrition *3*, 765, 1936.
9. Freudenberg, R.: Ueber die Verwendung von Vitamin B_1 und B_2 bei der Insulinschocktherapie der Schizophrenie. Wien. klin. Wochschr. *50*, 535, 1937.
10. Habenkamp, T.: Kopfschussverletzung und Vitamin B_1. Psychiat.-Neurol. Wochschr. *39*, 261, 1937
11. Bickel, G.: Essai de Traitement de l'Artérité Sténosante des Extrémités par la Vitamin B_1. Rev. méd. de la Suisse Rom. *57*, 321, 1937.

12. Vorhaus, M. G.: The Present Evaluation of Vitamin B_1 Therapy. Am. J. Digest. Dis. and Nutrition *3*, 915, 1937.
13. Meiklejohn, A. P.: Estimation of Vitamin B_1 in Blood by a Modification of Schopfer's Test. Biochem. J. *31*, 1441, 1937.
14. Cline, J. K.: Personal communication.
15. Wells, A. H.: The Preparation of Tikitiki Extract for the Treatment of Beriberi. Philip. J. Sci. *19*, 67, 1921.

CHAPTER VII

HUMAN DIETS IN RELATION TO BERIBERI

THE character of treatment in this chapter and the succeeding one will present a marked contrast to that of the preceding ones. The reason for this is that we here have to present a new standard for the appraisal of diets which has not been published elsewhere. Accordingly, we have felt it desirable to present the evidence in some detail.

The new tentative standard has arisen out of the re-examination of the voluminous data collected and scrutinized by Cowgill as a means of verifying his formula. In a recent publication we ventured the prediction that his data would prove an excellently conceived basis for a reappraisal of the vitamin B_1 requirement of man (1). At that time we scarcely hoped to participate so soon in the fulfillment of the prediction.

Cowgill (2) has analyzed 180 human dietaries which have been historically associated with beriberi or with its absence among particular groups of people. Included among these are a large number which represent minor variations of one another, such as optional allowances of lesser components of the diet. A further number is made up of diets which were consumed alternately on successive days. Eliminating such minor variations by combining them into a composite typical of each group of variants, the net number of diets analyzed is about 100.

In analyzing these diets Cowgill adopted the principle that the vitamin-calorie ratio required rises with the weight of the individual. He found that with the exception of ten diets the analyses fell in fairly well with his predictions. However, we feel that the principle of the rising requirement as to vitamin-calorie ratio is controverted by the prevailing evidence (see Chapter XXIII).

To leave this principle out scarcely spoils the fit of Cowgill's figures to the facts about incidence or non-incidence of beriberi. He did not make a comparison of the entire list of beriberi-producing diets with a similar list of the non-beriberi-producing diets but merely compared them in small groups which were historically associated with one another. A comparison of Cowgill's values for the entire category of the one sort with the entire category of the other sort is brought out in the following table:

CLASSIFICATION OF DIETS BY COWGILL'S RATING

Vitamin/Calorie	*Beriberi*	*Non-Beriberi*	*Total*
0.46–1.69	50	0	50
1.70–1.99	9	8	17
2.00–2.29	6	12	18
2.30–	1	14	15
			100

It is to be noted that there is a rather wide range of vitamin/calorie values in which appear both beriberi- and non-beriberi-producing diets in similar proportions.

All of these diets have been reanalyzed, using the thiamin contents taken from the tables in Chapter XVIII. In the following table is given, under the column "T/Calories," micrograms (γ) of thiamin in the diets divided by calories. We use the symbol "T" to distinguish our own estimates of thiamin content from those of Cowgill, which are designated "vitamin" in accordance with his practice.

CLASSIFICATION OF DIETS BY THIAMIN-CALORIE RATIO

T/Calories	*Beriberi*	*Non-Beriberi*	*Total*
.074–.229	52	1	53
.230–.249	9	2	11
.250–.279	2	2	4
.280–	3	29	32
			100

If one wishes to compare Cowgill's absolute values with our own, the former may be converted into terms of the latter by multiplying by 0.20, the value in the terms of γ of thiamin of the milligram equivalent unit used by Cowgill. His dividing line between the predominantly beriberi-producing diets and the beriberi-preventing ones is about Vitamin/calories $= 2.0$, or in terms of γ, Vitamin/calories $= 0.4$. This is much higher than our value, T/calories $= 0.25$, for the dividing line. The discrepancy is principally due to our preference for values of thiamin content based upon the cure or prevention of polyneuritis, while Cowgill used anorexia prevention values. The foods chiefly concerned with the discrepancy in these diets are beef, liver, molasses, and several vegetables which Cowgill rated higher than we do. He also assigned vitamin values to fats as we have not. Further we have made a larger allowance for losses in sprouting and cooking of beans than he did. All these matters involve large elements of judgment concerning which much could be said pro and con. We feel, however, that the sharp dividing line between beriberi-producing and beriberi-preventing diets, as shown in the above table, is a reasonable defense of our choices of values of thiamin content. For if the assigned values were too high for some foods and too low for others, the relative positions of the several diets would be displaced.

In Table II we have arranged the diets in the ascending order

TABLE II

Reanalysis of Beriberi-Producing and Beriberi-Preventing Diets

From Cowgill

Designation of Diet	Cowgill Page	Thiamin per Day	Calories per Day	T / Cal.	Vit. per Day Cowgill	Vitamin / Calories Cowgill	Fat Cal.	T / N.F.C.*	Extent of Beriberi
1. Aykroyd #3 B	109	225	1974	0.074	3022	1.53	270	0.082	B
2. Aykroyd #13 B	109	300	2480	0.078	3871	1.56	234	0.116	B
3. Aykroyd #2 B	109	308	2520	0.079	3901	1.55	238	0.116	B
4. Strong and Crowell IV	124	268	2310	0.099	1160	0.82	360	0.153	XXXX
5. Selangor Jail 1892	127	256	2581	0.099	1190	0.46	24	0.101	XXX
6. East Indian Navy Natives (fish)	149	420	3810	0.110	2950	0.77	171	0.115	XXXX
7. Frazer and Stanton I	120	346	3060	0.113	2599	0.85	567	0.223	XXX
8. Indian Troops Trincomalee (1900)	163	446	3922	0.114	3907	1.00	540	0.132	XXX
9. Singapore Prison 1869–75	134	336	2952	0.114	2010	0.68	261	0.125	XXX
10. Strong and Crowell II	124	268	2310	0.115	1940	0.84	347	0.137	XXX
11. Selangor 1901 Ordinary	127	402	3150	0.128	2865	0.91	502	0.152	XXX
12. Kuala Lumpur I	118	443	3379	0.131	3224	0.96	315	0.141	XXX
13. East Indian Native Sailors 1874	149	565	4208	0.134	5450	1.30	455	0.157	XXX
14. Selangor Jail, Penal, 1902	127	284	2084	0.136	1418	0.68	18	0.137	XXXX
15. Bilibid Prison 1901–02	139	368	2661	0.138	2618	0.98	261	0.153	XXXX
16. Selangor Jail, Penal, 1900	127	306	2193	0.140	1532	0.70	20	0.141	XXXX
17. Negro Laborers Congo I	178	475	3295	0.144	3200	0.97	118	0.154	XXXX
18. Singapore Prison 1876	134	342	2315	0.148	1873	0.81	63	0.152	XXX
19. Philippine Scouts 1908	165	578	3908	0.148	4151	1.04	389	0.164	XXX
20. Java Prisons (fish)	143	420	2831	0.148	2690	0.95	64	0.152	XX
21. Java Prisons (dried meat)	143	440	2927	0.150	2930	1.00	85	0.150	XX
22. Madras Native Troops (rice)	161	573	3804	0.151	4341	1.14	180	0.158	XX
23. Dutch E. Ind. Navy, Native 1874 (fish)	149	600	3960	0.151	4000	1.01	144	0.157	XXX
24. Mediterranean Troops A1	170	672	4400	0.153	4280	0.97	1220	0.211	XX
25. Pudah Gaol	116	487	2970	0.164	4013	1.35	453	0.193	XX
26. Aykroyd #1 B	109	442	2700	0.164	4037	1.44	272	0.182	B
27. Kut-el-Amara British Jan. 22	172	469	2839	0.165	3944	1.39	747	0.224	XXX
28. Aykroyd #11 B	109	934	5530	0.169	8677	1.57	1422	0.227	B

Designation of Diet	Cowgill Page	Thiamin per Day	Calories per Day	T / Cal.	Vit. per Day Cowgill	Vitamin / Calories Cowgill	Fat Cal.	T / N.F.C.*	Extent of Beriberi
29. Lascar Seamen	180	670	3956	0.170	6378	1.67	729	0.208	X
30. Dutch E. Ind. Navy Natives 1878	152	948	5534	0.171	9066	1.64	990	0.187	X
31. Java Prisons (beef and pork)	143	550	3211	0.171	3930	1.22	315	0.184	XX
32. Aykroyd #9 B	109	818	4720	0.173	6992	1.48	558	0.196	B
33. Aykroyd #8 B	109	676	3870	0.175	6819	1.76	602	0.206	B
34. Selangor 1902 Ordinary	127	482	2690	0.175	3010	1.12	504	0.220	X
35. Japanese Ryujo Marines	156	521	2875	0.181	3453	1.20	154	0.192	XXXX
36. Bilibid 1902	140	478	2622	0.182	3386	1.29	308	0.207	X
37. Aykroyd #2 N.B.	109	629	3430	0.183	7281	2.12	640	0.225	No
38. Selangor 1899 Ordinary	127	561	3032	0.185	3360	1.11	517	0.222	XXX
39. Aykroyd #4 B	109	495	2660	0.186	5076	1.91	324	0.212	B
40. Selangor 1900 Ordinary	127	468	2454	0.190	2410	0.98	396	0.227	XXX
41. Singapore Prison July 1880	134	535	2720	0.197	4303	1.58	48	0.200	XX
42. Aykroyd #5 B	109	727	3665	0.198	7924	2.16	640	0.240	B
43. Aykroyd #6 B	109	576	2860	0.201	6794	2.37	567	0.252	B
44. Aykroyd #12 *B*	109	557	2740	0.203	4471	1.63	334	0.231	B
45. Singapore Prison 1900	134	645	3128	0.206	4140	1.32	125	0.215	XXX
46. Mediterranean Troops A2	170	918	4400	0.208	6700	1.52	1096	0.278	XX
47. Singapore Prison 1897	134	624	3005	0.208	4140	1.38	149	0.218	X
48. Dutch E. Ind. Navy, 1874 Europeans	150	1091	5189	0.209	10441	2.05	1544	0.298	X
49. Aykroyd #10 B	109	632	3020	0.209	5467	1.81	350	0.236	B
50. Selangor 1895 Ordinary	127	618	2942	0.210	4240	1.44	522	0.255	XX
51. Japan. Ship Ryujo Cadets	156	651	3072	0.212	4450	1.45	387	0.242	XXX
52. Aykroyd #7 B	109	510	2400	0.212	4450	1.85	530	0.273	B
53. Megaw and Bhattacharjee Parsibazar	113	464	2100	0.220	4116	1.96	68	0.228	B
54. Megaw and Bhattacharjee (Campbell)	113	506	2200	0.230	4640	2.11	191	0.251	B
55. Ryujo Sub-officers	156	660	2867	0.230	4562	1.59	531	0.282	XX
56. Strong and Crowell III	125	442	1916	0.231	3060	1.60	324	0.278	XX
57. Jap. Navy Food Act. 1884	157	995	4295	0.232	6693	1.56	666	0.274	X
58. Ryujo Officers	156	819	3508	0.233	6164	1.76	742	0.296	X

TABLE II — *Continued*

Designation of Diet	Cowgill Page	Thiamin per Day	Calories per Day	T / Cal.	Vit. per Day Cowgill	Vitamin Calories Cowgill	Fat Cal.	T / N.F.C.*	Extent of Beriberi
59. Kala Bagan	113	432	1822	0.237	3966	2.18	54	0.243	X
60. Aykroyd #7 N.B.	109	751	3160	0.238	6719	2.13	380	0.270	No
61. Selangor, Penal, 1895	127	646	2720	0.238	4160	1.53	47	0.242	XXX
62. Selangor, Penal, 1893	127	710	2982	0.238	4560	1.53	47	0.241	X
63. Singapore Prison 1898–99	134	581	2424	0.240	4000	1.65	153	0.295	XXX
64. Bengali Girls	114	532	2200	0.242	4980	2.26	410	0.297	No
65. Singapore Prison Sept.–Dec. 1881	134	904	3475	0.260	6460	1.86	295	0.284	XXX
66. Singapore Prison 1882–85	134	970	3652	0.266	7050	1.93	324	0.294	X
67. Aykroyd #1 N.B.	109	703	2590	0.271	5418	2.09	504	0.336	No
68. Selangor 1892	127	1034	3766	0.274	7040	1.87	419	0.309	No
69. Mediterranean Troops	170	1013	3620	0.280	6990	1.93	570	0.321	No
70. Magaw and Bhattacharjee Hindus	114	616	2295	0.281	5643	2.46	56	0.275	No
71. Aykroyd #3 N.B.	109	764	2710	0.282	5587	2.09	414	0.332	No
72. Mediterranean Troops B	170	1033	3590	0.288	6200	1.73	855	0.378	No
73. Aykroyd #10 N.B.	109	1035	3690	0.288	6821	1.85	495	0.320	No
74. Aykroyd #11 N.B.	109	875	2945	0.297	5194	1.76	471	0.353	No
75. Aykroyd #4 N.B.	109	820	2710	0.300	5722	2.11	781	0.424	No
76. Aykroyd #9 N.B.	109	1497	4980	0.300	10264	2.06	900	0.367	No
77. Selangor 1893–94 Ordinary	127	965	3142	0.308	6880	2.19	670	0.390	No
78. Aykroyd #5 N.B.	109	1664	5510	0.302	10754	1.95	864	0.357	No
79. Megaw and Bhatt. Anglo-Ind.	113	932	3000	0.310	6373	2.12	670	0.281	X
80. Aykroyd #12 N.B.	109	1597	4980	0.321	10266	2.06	1062	0.407	No
81. Bilibid Prison 1912 Manila	141	941	2911	0.324	5779	1.97	176	0.345	No
82. Kut-el-Amara Indians March 11	172	726	2208	0.328	4592	2.31	540	0.434	No
83. Richmond Asylum-Dublin 1897	145	982	2994	0.328	6002	1.93	420	0.382	XXX
84. Kut-el-Amara British March 11	172	771	2304	0.334	5660	2.46	695	0.478	No
85. Garrison, San Juan Jan.–Apr.	175	600	1718	0.350	4198	2.22	221	0.401	X
86. Megaw and Bhatt. Mohammedans	114	778	2180	0.357	5117	2.35	58	0.366	No
87. Aykroyd #6 N.B.	109	954	2580	0.370	9023	3.50	621	0.487	No
88. Indian Native Troops (Improved)	163	1500	4025	0.373	15728	3.90	352	0.409	No
89. Kut-el-Amara British Feb. 10	172	1063	2835	0.375	4931	1.74	779	0.517	No

Designation of Diet	Cowgill Page	Thiamin per Day	Calories per Day	T / Cal.	Vit. per Day Cowgill	Vitamin Calories Cowgill	Fat Cal.	T / N.F.C.*	Extent of Beriberi
90. Singapore Prison 1885–1896	134	585	2514	0.385	5630	2.24	288	0.435	No
91. Kut-el-Amara Indians Mar. 4	172	983	2149	0.457	5232	2.41	550	0.614	No
92. Aykroyd #8 N.B.	109	2070	5020	0.413	12299	2.45	630	0.472	No
93. Kut-el-Amara Indians Jan. 22	172	1217	2843	0.428	8706	3.06	620	0.548	No
94. U. S. Garrison San Juan June	175	866	1882	0.460	6125	2.77	346	0.562	No
95. Kut-el-Amara British Mar. 4	172	1168	2420	0.481	5924	2.45	700	0.679	No
96. Philippine Scouts after 1911	169	1770	3672	0.482	5802	1.58	408	0.542	No
97. Trincomalee Troops 1901	163	2194	4200	0.522	14131	3.36	775	0.657	No
98. Frazer and Stanton 1907–08	120	1695	3054	0.555	7673	2.51	504	0.664	No
99. Kuala Lumpur Asylum	118	2170	3381	0.624	6114	1.81	300	0.707	No
100. Ind. Native Troops Attah	161	3925	3994	0.985	23729	5.94	495	1.121	No

Thiamin is given in γ per gram; Cowgill's vitamin values in milligram equivalents (0.2 γ). Prevalence of beriberi is indicated by numbers of X's, except when the number of cases is not recorded or the group was too small for statistical consideration, in which case the existence of beriberi is indicated by B.

* Non-fat calories.

of their thiamin-calorie values to facilitate a comparison of these values with those of vitamin-calories. Taking the diets individually, there are many substantial discrepancies in the numerical equivalence of the two ratios. However, looking at the series of diets as a whole, there is a general conformance, that is, there are relatively few diets for which the two methods would lead to contrary predictions as to the occurrence or non-occurrence of beriberi. There is also correspondence in the fact that neither "vitamin per day" nor "thiamin per day" furnishes a good index of the incidence of the disease, whereas either the ratio vitamin-calories or thiamin-calories does so to a very significant degree. This further justifies Cowgill's proposition that the vitamin required is related to the amount of food consumed. For further discussion of this matter see Chapter XX.

For these diets, the thiamin-calorie ratio turns out to be an excellent index of the probability of beriberi. There are only three diets (Nos. 37, 60, 64) of a thiamin-calorie ratio less than 0.25 which failed to produce beriberi and only five diets (Nos. 65, 66, 79, 83, 85) of a higher ratio than those which are recorded as having produced beriberi. Of these eight discordant diets, four have values very close to the dividing line. The remaining four are ones which are discordant with any proposed index. Probably the recorded information about these diets is defective or erroneous in some significant particular. It should be said that in general the original data available about most of the diets referred to in the table are meager or only approximate in character, so that the degree of consistency obtained is rather surprising.

While there is no reason on the face of these figures for mistrusting the thiamin-calorie ratio as an index to beriberi-producing quality, there are other reasons for seeking a different index. In Chapter XXI there is reviewed the evidence regarding the effect of the proportions of carbohydrate, protein, and fat upon the vitamin B_1 requirement of animals. The essence of the findings is that the requirement rises with the amount of carbohydrate consumed but falls with increasing proportions of fat in the food. The effect of proportion of protein is not so clear. Accordingly we experimented with the thiamin-carbohydrate ratio and the thiamin-non-fat-calorie ratio of the diets. The thiamin-carbohydrate ratio did not prove at all satisfactory as an index, as indicated in the following summary:

Classification of Diets by Thiamin-Carbohydrate Ratios

T/Carbohydrate	*Beriberi*	*Non-Beriberi*	*Total*
0.58–1.20	51	3	54
1.20–1.70	11	10	21
1.70–2.50	5	12	17
2.51–5.70	0	8	8
			100

The thiamin-non-fat-calorie ratio proved to be fully as good an index for the diets in Table II as the thiamin-calorie ratio, in fact somewhat better. Three diets (Nos. 37, 83, and 85) are highly discordant, but they are ones which are discordant with any index and probably reflect faults in the original dietary data. Three other diets (Nos. 60, 64 and 70) are slightly discordant but not sufficiently to cause great concern. The borderline is clearly at or near a ratio of 0.3, as seen below:

Classification of Diets by Thiamin-Non-Fat-Calorie Ratio

T/N.F.C	*Beriberi*	*Non-Beriberi*	*Total*
.082–.250	50	1	51
.251–.300	13	4	17
.301–.350	0	6	6
.351–	2	24	26
			100

The thiamin-non-fat-calorie ratio is not precisely ideal theoretically because experiments with animals have shown that the natural fats from different sources are not fully equivalent to one another in "vitamin B_1-sparing" action (see pages 266–276). However, practically it is felt that in any mixed diet there will be a sufficient diversity in the fats which are present so that the "sparing" action can be treated as being moderately effective in all diets. As we shall see in the next chapter, the thiamin-non-fat-calorie ratio provides a much more reasonable picture of Occidental diets than the thiamin-calorie ratio. Indeed the latter ratio would lead to a prediction of widespread beriberi in the United States, where it rarely occurs in severe form, while the former does not.

Cowgill recognized the desirability of compensating for the fatty components of foods and took care of the matter by assigning a value of thiamin content to each fat or fatty food. Experience which has accumulated since his book was written points more and more to the complete absence of thiamin in strained fats and negligible amounts in the tissues of fat depots. His expedient is, therefore, theoretically objectionable, though it served to a large degree and in an empirical way the purpose which he had in mind.

With regard to the fact that the thiamin-carbohydrate ratio proved unsuitable, whereas many animal experiments had suggested the contrary, it should be remarked that these experiments all involved diets of very low fat content and for the most part diets of relatively low protein content. They were not, therefore, well planned to detect the equivalence of protein and carbohydrate in respect to their effect on thiamin requirement. Abderhalden and Wertheimer (3) have, however, taken the stand, based on experiments with pigeons, that proteins must be regarded as "carbohydrate builders" in calculating the thiamin re-

quirement. The superiority of T/N.F.C. to T/carbohydrate as an index of beriberi production is fully in accord with their view, a view which has had as yet little support from other quarters. We shall, therefore, commit ourselves to the thiamin-non-fat-calorie ratio as the best tentative index of adequacy for protection of the average individual that can be offered at present. We entertain considerable hope that it may gain respect as it comes to be tried by others. However, it is realized that we know very little about the possible influence of many factors upon the vitamin B_1 requirement of man. Not only are there questions of the effects of adequate or inadequate supplies of minerals or other vitamins in the food but also complications of physical hardship, existence of infections, etc. (see pages 85 and 86). We have no expectation, therefore, that any index will prove invariably applicable where subclinical manifestations of deficiency are involved.

There are some other possible ways of arriving at the thiamin requirement of man. Jolliffe and Goodhart (4) have made a beginning with the method of direct experimentation. They found that four of the five individuals with whom they have experimented to date showed distinct signs of deficiency after only a week or ten days on a partially deficient diet. The signs noted included anorexia, fatigue, dyspnea, precordial pain, calf pain, solar hyperesthesia, and slight electrocardiographic changes. By carefully following the excretion of vitamin B_1 in the urine, both prior to and during the depletion period, as well as in a post-experimental period during which substantial known amounts of the synthetic vitamin were given, correlative data were obtained which add greatly to the seeming trustworthiness of the observations.

Another possible approach to the human requirement is via the composition of human milk, for it must be assumed that normally fed mothers will on the average excrete milk which is adequate for their young. However, our knowledge of the vitamin B_1 content of human milk is not in a very satisfactory state. (See Chapter XXII.) Such information as exists indicates a content substantially lower than that of cow's milk, but further confirmation is required. A very useful study in this connection which is yet to be done is that of ascertaining how the thiamin content of the milk varies with the administration of known amounts of thiamin chloride to the mother. Even if the thiamin content of the milk were known with precision, it would remain debatable as to how well the requirement of nursing young would tally with that of the adult.

If our preferred values are substantially correct (we admit the necessity for their further verification), the thiamin-non-fat-calorie ratio necessary to protect against beriberi is very close

to 0.3. Assuming certain proportions of fat in a diet of 2500 calories per day, we obtain the following figures by way of illustration:

Fat Calories	*Non-Fat Calories*	*Thiamin γ per Day*
0	2500	750
500	2000	600
700	1800	540
1000	1500	450

German authorities (5) regard the minimum requirement of man as lying between 250 and 750 γ per day and quote the Hygiene Section of the League of Nations as supporting a value of 300 International Units per day. This quantity the Germans equate to 600 γ, but it can be more reasonably taken as 1200 γ and is certainly not to be construed as less than 900 γ. This League of Nations estimate, however, is intended as a safe minimum for health and not merely for protection against beriberi. Van Veen (6), who is in touch with the Dutch experience in the East Indies, estimates that 150 I.U. per day, which he equates, correctly as we think, to 600 γ, represents the minimum necessary for protection against beriberi; 200 I.U. or 800 γ is regarded as a safe level for Orientals. Some British authorities are inclined to put the requirements for protection somewhat higher, viz., at 200–500 I.U., which, according to the conversion factor used by them, should be taken as equivalent to 600 to 1500 γ (7), while the German authorities above cited express the opinion that 1 to 2 mg. (1000–2000 γ) are required for health. We consider the latter estimate to be substantially correct for the average person, the higher level being required for persons who perform considerable physical work and accordingly consume 3000 to 4000 calories per day. As discussed in Chapter VI, some individuals may well require still larger amounts. The confusion regarding conversion factors for International Units is discussed on pages 213–216.

The question of milling of grain is of prime importance for Oriental peoples in rice-eating areas, where the diet is much less varied than in the West. There can be no question that for good nutrition in all respects these Orientals need a more varied diet with a higher proportion of high-protein and so-called protective foods. To take the view, however, that the Orient must continue to suffer from beriberi until such a revolution in economic status is accomplished is well-nigh a counsel of despair. We regard it as amply demonstrated that the sole substitution of unpolished rice for polished would serve to eradicate most of the outright beriberi in the world and enormously improve the vigor of Oriental peoples.

A prohibitive tax on polished rice has been frequently pro-

posed (8) but has not been put into effect. Difficulties were involved in lack of a wholly suitable means of assaying rice for its vitamin content. These have now been overcome sufficiently for practical purposes. Methods such as those used by Spruyt (9) in his excellent study of 184 varieties of rice would undoubtedly serve. Other objections still exist. Among these is the prejudice of the people against brown undermilled rice, a prejudice quite well paralleled in the West in reference to dark bread. Some of the existing objections to taxing rice are quite practical, and it will require considerable experimentation and development, as well as a great deal of public promotion, to overcome them. They have to do with the greater tendency of undermilled grain to spoilage by weevils and by the development of rancidity. Compared, however, with the problem of sanitary distribution of milk, these difficulties are not insurmountable. A light roasting or parboiling of the grain would serve to destroy enzymes causing rancidity and to kill the eggs of beetles. Transport and storage of the grain in paddy form so as to secure the protection afforded by the closely adherent husks until shortly before its milling and consumption would probably be helpful.

Another expedient can now be considered, that of incorporating the synthetic or natural vitamin in the diet. Its incorporation in polished rice in such a way as to prevent its complete removal in washing prior to cooking would be difficult. Aykroyd has shown that the vitamin is caused to penetrate the grain during the parboiling of paddy, but such a process carried out at commercial centers remote from points of consumption would offer new hazards of spoilage in transit and storage. A more feasible expedient at present appears to be the incorporation of synthetic vitamin in the domestic salt supply. This would necessarily increase the price of salt greatly, but since this commodity is a very minor item in the family budget, its cost would not be intolerable. Government regulation of the matter would probably be necessary in the Orient. Something of the order of 1 gram of thiamin per kilo of salt would suffice, and the universality of the use of salt in the cooking of rice would insure a reasonably uniform distribution of the vitamin among the population. At all events, some such expedient can doubtless be found which would eradicate beriberi long before a complete economic revolution can be hoped for.

BIBLIOGRAPHY

1. Williams, R. R.: Chemistry of Thiamin. Ergebnisse der Vitamin- und Hormonforschung, *1*, 227, 1938.
2. Cowgill, G. R.: Vitamin B Requirement of Man. Yale University Press, New Haven, 1934, p. 183.
3. Abderhalden, E., and Wertheimer, E.: Beziehungen des Vitamin-B-Komplexes (insbesondere des Vitamins B_1) zum Kohlehydrathaushalt. Arch. ges. Physiol. (Pfluger's) *233*, 395, 1933.
4. Joliffe, N., and Goodhart, R.: The Urinary Execretion of Vitamin B_1. Its Dependency on Dietary Intake in Normal Subjects. Proc. Am. Physiol. Soc. 1938, p. 111.
5. Stepp, W., Kuhnau, J., and Schroeder, H.: Die Vitamine und ihre klinische Anwendung. F. Enke, Stuttgart, 1937, p. 42.
6. Van Veen, A. G.: Het B_1 gehalte der voornaamste Indische voedingsmiddelen. Geneesk. Tijdschr. Ned. Ind. *75*, 2050, 1935.
7. Baker, A. Z., and Wright, M. D.: Vitamin B_1 in Human Diets. Proc. Roy. Soc. Med. *29*, 1145, 1936.
8. Vedder, E. B.: Beriberi. Wm. Wood, New York, 1913, pp. 317–328.
9. Spruyt, J. P.: Een chemische methode voor de keuring van rijst op het gehalte aan antineuritisch (B_1) vitamine. University of Amsterdam Thesis, 1933.

CHAPTER VIII

OCCIDENTAL DIETS

We have considered in the preceding chapter diets associated with clinical beriberi. As beriberi is, for the most part, an Oriental disease, it is of interest to survey the diets of the Occident to develop an appreciation of the reasons which underlie the prevailing geographic distribution of this disease. Recognizing that beriberi does occur elsewhere than in the Orient, it is nevertheless true that its occurrence in epidemic proportions is largely limited to Eastern countries. Why should this be so, and what is the margin of safety against beriberi which the Western world enjoys?

Some years ago it was commonly supposed that there was a great gulf, with respect to thiamin content, between the Oriental diets associated with beriberi and those of Western peoples who were almost free from the disease. More and more doubt has been thrown upon the correctness of this view, but opinion is still divided. One group has taken the view that the varied diets typical of the West constitute a blanket protection of the populations against a deficiency of this vitamin. For example, Aykroyd (1) states "there is really little reason to believe that the average English or American dietary, whether it includes whole meal bread or not, contains insufficient vitamin B_1 for health. Fortunes have been made in recent years out of the sale of 'Vitamin B' preparations in tins with attractive labels." The author goes on to state, "The more a population relies on a single cereal for food and the smaller the quantity of non-cereal foods in the diet, the greater becomes the importance of the degree of milling to which the staple cereal is subjected. While much nonsense has been talked about the deficiency of vitamin B in our modern city dietary, there are times when it seems wise to insure an abundance of it, perhaps by the consumption of whole meal bread."

At the other extreme is the view that seeds constitute the principal repository of thiamin in the plant world and that whenever man practices the mechanical separation of the starchy part of the seed from the germ and bran coats, he is endangering the adequacy of his thiamin supply unless his total carbohydrate intake is low.

Another group takes an intermediate view. They hold upon the basis of general observation and experience that there is a widespread liability to vitamin B_1 deficiency. This group includes: Physicians who frequently see patients with clinical signs of a deficiency of vitamin B_1 (see pages 4, 5) and students of nutrition who have studied the dietaries in large numbers of families. For example, in reporting a study of the nutritive value of the diets of families in the North Atlantic states, Stiebeling (2) states, "Of the nutrients considered, protein appears to be the one most abundantly furnished with reference to need, and calcium, iron and vitamin B, the least."

A dietary survey on a large number of American dietaries in various sections of the United States (3) gives general support to the view that the American diet may fail to supply an adequate amount of vitamin B_1. It will be noted that in most instances in the following tabulation (Table III) the ratio, thiamin/calories, falls near or below the beriberi borderline. In two instances the thiamin/non-fat-calories also closely approaches it and, in a third, actually sinks below it, presumably an evidence of a defect at some point in our procedure or in the record of facts. While the latter ratio cannot be accepted with finality as the correct index of thiamin adequacy, it appears to be a sub-

TABLE III

VITAMIN B_1 CONTENT OF DIETS OF FAMILIES OF WAGE EARNERS AND LOW-SALARIED CLERICAL WORKERS (4)

REGION, NUMBER OF FAMILIES	WEEKLY EXPENDITURE PER FOOD CONSTITUENT	THIAMIN PER DAY	CALORIES PER DAY	$\frac{T}{Cal.}$	FAT CALORIES	NON-FAT CALORIES	$\frac{T}{N.F.C.}$
North Atlantic, 1394 white families	$1.33–$1.99	600	2550	.235	601	1949	.308
	2.00– 2.66	735	2960	.248	717	2243	.329
	2.67– 3.32	885	3310	.276	915	2395	.370
	3.33– 3.99	945	3840	.246	989	2951	.320
	4.00– 4.66	1200	4140	.290	1165	2975	.404
Pacific: 688 white families	1.33– 1.99	780	2570	.303	705	1765	.442
	2.00– 2.65	840	3100	.271	785	2315	.363
	2.67– 3.32	960	3660	.262	975	2685	.357
	3.33– 3.99	1065	4140	.257	1095	3045	.349
	4.00– 4.66	1125	4340	.259	1325	3015	.373
East South Central: 426 white families	.67– 1.32	495	2620	.189	685	1935	.256
	1.33– 1.99	735	3050	.241	800	2250	.327
	2.00– 2.66	855	3470	.246	990	2480	.344
	2.67– 3.32	1095	3980	.275	1185	2800	.391
South: 284 Negro families	.67– 1.32	570	2450	.232	660	1790	.318
	1.33– 1.99	840	3460	.243	1070	2390	.351
	2.00– 2.66	1180	4470	.242	1280	3190	.339
	2.67– 3.32	1260	4880	.259	1445	3435	.367
Sherman — Average Amer. Diet (Cowgill p. 186)		900	2500	.360	830	1670	.539
American Family on Food Relief (Cowgill p. 194)				.400			.584
Assumed borderline for clinical beriberi		600	2500	.250			.300

Sherman units of the original table were converted to thiamin by multiplying by 1.5.

stantially better index for the great majority of typical mixed diets of the Western world than any other which is now available. Clearly the simple thiamin-calorie ratio cannot apply to people on these diets. If it did, there would be a significant incidence of forthright beriberi among them. Viewed in the light of all the facts, the poorer populations of the United States appear to owe their freedom from beriberi as much to the relatively liberal fat content of their food as to a greater liberality of thiamin supply, as compared with the people of the Orient. Such a favorable effect of fat in the diet is well borne out by animal experiments (see Chapter XXI).

Although due allowance must be made for possible errors in estimating the thiamin content of foods and for errors in converting Sherman Units to γ of thiamin, the data in Table III show that for the average person, many of these diets afford very little margin above any definable borderline for beriberi, so much so that one might expect an appreciable incidence of beriberi in such families. Clinical studies on fifty severe endemic pellagrins showed that thirty-one had some evidence of peripheral neuritis. This neuritis improved following the administration of large amounts of yeast, and presumably vitamin B_1 deficiency played a role in the development of the polyneuritis (see page 45). It must be borne in mind that studies of the dietaries of families or groups of persons sharing a common food supply reveal only the average intake. To determine how satisfactory a diet is for an individual, we should know the kind and amount of food each consumes. For many reasons an individual may fail to eat his theoretically allotted share of the group food supply. There are many recorded instances in the literature in which one member of the family has contracted clinical forms of a deficiency disease because of individual idiosyncrasies. There is little information available on the dietaries of individual members of a family or group, or on the dietaries of persons who are not members of a household or group. Studies of this kind should be made.

As additional evidence regarding thiamin content of American dietaries and the significance of the two ratios which have been discussed, we also show in the above table our analysis of two relatively good diets which Cowgill has discussed.

A striking fact about Occidental diets is that they are not immeasurably superior to those of the East in absolute thiamin content. As compared with some of the diets which Cowgill has pointed out as associated with beriberi, the thiamin contents in general are less than doubled. This is brought out in the following brief comparison of certain selected diets (see Table IV). It is rather conspicuous that the richest diets of the world with respect to the thiamin content, as well as the poorest diets, often

contain a high percentage of grain. The degree of decortication of the grain is often all-important in such cases. The two diets used by Fraser and Stanton in their classical experiments illustrate this point forcibly.

TABLE IV

Comparison of Oriental and Occidental Diets

Diet	*Daily Thiamin Intake, Mgs.*	
	Cowgill's Values	*Authors' Values*
Borderline for beriberi in starchy diets	1	.6
Selangor Jail 1895 Penal	.76	.59
Ryujo Cadets	.72	.53
Fraser and Stanton I (polished rice)	.43	.29
Fraser and Stanton II (parboiled rice)	1.25	1.39
Sherman's Average American Diet	1.37	.90
American Family on Food Relief	1.35	1.00

It is assumed that 2500 calories a day were consumed in all cases.

More and more the opinions of students of nutrition appear to be veering toward attaching great importance to decortication of grain. A noteworthy paper which emphasizes this viewpoint is that of Baker, Wright, and Drummond (4). These authors traced the progress of the increasing use of white flour to the introduction of the silk bolting cloth in 1840 and of roller mills in 1870 to gratify a popular demand. They estimate that, as a consequence of the progressive milling of wheat, "the best fed members of the population today are getting twice as much vitamin B_1 as people on a low income level, yet consume less vitamin B_1 than the parish poor of the eighteenth and earlier nineteenth centuries."

There can be no doubt that the use of varied diets is conducive to health. The so-called protective foods undoubtedly contribute essential mineral elements as well as necessary vitamins to the diet, but we feel that the importance of these protective foods with respect to thiamin intake has been considerably exaggerated in the minds of students of nutrition.

As pointed out elsewhere (Chapters XVIII and XXVIII), thiamin is present in virtually all tissues of plants or animals which are used as food. However, the proportions are prevailingly very small, that is, less than one part per million. In striking contrast to the distribution of vitamin A, there are no super-rich sources of thiamin among the common foodstuffs, so that it is not possible by occasional serving of a particular food to make up for the deficiencies of the major components of the diet. Yeast and wheat germ are now sometimes used as such reinforcements but cannot be classified as foods in the ordinary sense. The classification of foods into three categories in the manner first proposed by Jansen is excellent (5):

I. The thiamin-enriching foods, under which we include whole seeds, pork, milk, liver, heart, and kidney.

II. The diluents, notably white flour, polished rice, macaroni, spaghetti, white breakfast cereals, cane sugar, and molasses.

III. The neutral, or indifferent, sources, which include meats (other than pork and internal organs), fresh fruits, and vegetables. These are sufficiently low in thiamin, or, because of their high water content, are consumed in relatively small quantities on a dry-weight basis, so that they do not alter greatly the thiamin-calorie ratio of the diet. They are, of course, much superior to added quantities of the diluent class.

In the light of recent studies the fats and fatty foods should receive special consideration as a fourth class.

At the present time the uncertainty with regard to the actual thiamin content of Occidental diets is of the same order of magnitude as any possible difference between Western and Eastern diets. It is impossible to affirm or to deny conclusively the proposition that the average Western diet contains adequate thiamin for good health. Certainly it must be more adequate than the diets of those countries where beriberi is so prevalent, but there is in all probability a great difference between the level necessary for excellent health and that which is required for the prevention of clinical beriberi (see Chapter VI). We must await more information on this point before we can say whether or not public health demands the abandonment of decortication of grains, both in the East and in the West. The advent of a reliable chemical method of assay would promote greater certainty of knowledge with regard to the thiamin content of foods.

Appraisal of Particular Diets

Physicians, nutritionists, and other groups frequently feel the need of a practical and ready means of estimating the adequacy of diets in respect to their thiamin content. A rough estimate which may be of assistance in avoiding badly balanced rations may be made conveniently from Table V (page 112), which gives the thiamin content of a variety of foods in terms of the average serving. Opinion as to what constitutes an average serving may vary, but this variation probably is not much larger than the degree of uncertainty of the actual thiamin value of the particular specimen of the food under consideration. In the few instances in which large numbers of samples of any particular foodstuff have been examined carefully, there is much to indicate that a wide variation exists among the samples. The variation is presumably due to such factors as the variety of the species, the ripeness of seeds, maturity or freshness of vegetables, length of storage, character of commercial process of preparation, etc. The character of a given staple food which is currently available in the market may differ markedly from place to place and from time to time. On these differences are

superimposed those due to variation in the methods of preparation for the table (6).

The Thiamin Content of Therapeutic Diets

Given a sufficiently wide variety of foods from which to choose, enough money with which to purchase them, a knowledge of the essential components of an adequate diet, and the wisdom to apply this knowledge, there should be no difficulty in selecting a diet which provides an adequate amount of thiamin for normal needs. However, difficulty may arise if, for therapeutic purposes, it is necessary to reduce the food intake or to omit certain foods.

That many types of therapeutic diets are prescribed may be seen by glancing through the table of contents of any one of the textbooks on diet in disease. While it is beyond the scope of this book to examine a large number of these diets in regard to the thiamin they supply, a few of them have been evaluated. Table VI is a summary of the diets examined. Two of the diets are shown in detail in Tables VII and VIII.

In considering such diets it seems best to give weight to all possible criteria as we do not know which index is most trustworthy for diets of these unusual types. Accordingly the values for total thiamin intake, and for the two ratios as well, should be brought up to the minimum levels indicated in Table VI as desirable. If, in order to meet one of these, the minimum levels for the others are greatly surpassed, no harm will be done.

These particular diets demonstrate how inadequate a diet may be if the restriction imposed prevents the inclusion in the dietary of foods which are good sources of thiamin. It is sometimes impossible to improve the diet by food alone, and in such instances thiamin supplements should be prescribed. The two 1000 calorie diets demonstrate the need of careful selection of food in planning a restricted diet. If a wise choice of food is made, thiamin may, in some instances, be supplied in an adequate amount for normal needs and still keep the calories low. If an unwise choice of food is made, there is a certainty of shortage of thiamin. The failure of a patient to make a wise selection of food for his diet is frequently due to poor or insufficient dietary instruction. He should not be instructed on the basis of calorie value of food alone, nor should he be given only a list of foods he may not eat and a list of the foods he is allowed. He should be instructed to include in his daily diet an amount of those foods which will assure his having a diet adequate, not only in thiamin, but in all the essential nutrients.

In the diabetic diets, and the well-planned 1000 calorie diet, attention should be called to the improved thiamin value of the diets when whole wheat, rather than white bread, is used.

TABLE V

The Thiamin Value of Average Servings of Some Common Foods

Food	Thiamin * γ per Gm.	I.U. per Gm.	Approx. Size of Serving	Wt. of Serving in Gm.	Thiamin per γ Serving	I.U. Serving †
Bread						
Rye, whole grain	2.0	.60	1 slice 3½″ sq. ¼″ thick	25	50.0	15.0
Rye, made with flour, 40% extraction	.8	.24	1 slice 3½″ sq. ¼″ thick	25	20.0	6.0
Rye, made with flour, 94% extraction	1.7	.51	1 slice 3½″ sq. ¼″ thick	25	42.5	12.8
Wheat specified as "whole" or "cracked"	4.0	1.20	1 slice 3½″ sq. ¼″ thick	25	100.0	30.3
Wheat, brown, unspecified	2.5	.75	1 slice 3½″ sq. ¼″ thick	25	62.5	18.9
Wheat, whiter forms	.5	.15	1 slice 3½″ sq. ¼″ thick	25	12.5	3.7
Wheat, less white forms	1.0	.30	1 slice 3½″ sq. ¼″ thick	25	25.0	7.5
Cereals‡ and Flour§						
Barley, pearled	.5	.15	2 tablespoons	30	15.0	4.5
			(2 tablespoons cooked = ½ cup)	(105)		
Corn meal, old process, low grade	1.0	.30	3 tablespoons	30	30.0	9.0
			(3 tablespoons cooked = ⅔ cup)	(150)		
Corn meal, old process, better grade	1.5	.45	3 tablespoons	30	45.0	13.6
			(3 tablespoons cooked = ⅔ cup)	(150)		
Corn meal, new process	.5	.15	3 tablespoons	30	15.0	4.5
			(3 tablespoons cooked = ⅔ cup)	(150)		
Farina	.5	.15	3 tablespoons	30	15.0	4.5
			(3 tablespoons cooked = ⅔ cup)	(170)		
Macaroni	.5	.15	⅓ cup	30	15.0	4.5
			(⅓ cup cooked = ⅔ cup)	(85)		
Oats, rolled	3.0	.90	¼ cup	30	90.0	27.2
			(¼ cup cooked = ⅔ cup)	(170)		
Rice, polished, unwashed	.5	.15	2 tablespoons	30	15.0	4.5
			(2 tablespoons cooked = ½ cup)	(110)		
Rice, polished, washed	.3	.09	2 tablespoons	30	9.0	2.7
			(2 tablespoons cooked = ⅓ cup)	(110)		
Rice, red	.3	.09	2 tablespoons	30	9.0	2.7
Rice, unpolished	2.2	.66	2 tablespoons	30	66.0	20.0

Food	Thiamin * γ per Gm.	I.U. per Gm.	Approx. Size of Serving	Wt. of Serving in Gm.	Thiamin γ per Serving	I.U. Serving †
Rice, cured	2.5	.75	2 tablespoons	30	75.0	22.7
Rye flour	1.7	.51	2 tablespoons	18	30.6	9.2
Wheat flour, graham	3.3	1.0	2 tablespoons	18	59.4	18.0
Wheat flour, straight run	1.0	.30	2 tablespoons	18	18.0	5.4
Wheat flour, patent	.5	.15	2 tablespoons	18	9.0	2.7
Wheat flour, whole	4.0	1.21	2 tablespoons	18	72.0	21.8
Wheat germ	40.0	12.1	2 tablespoons	15	600.0	181.8
Wheat bran, varies with milling process						
Dairy Products						
Butter	0	0	1 tablespoon	15	0	0
Cheese	trace		1 ounce	30	trace	trace
Cream	.35	.10	¼ cup	60	21.0	6.3
Eggs, hen's, whole	1.0	.30	1 egg	50	50.0	15.1
Eggs, hen's, white	0	0	1 egg white	34	0	0
Eggs, hen's, yolk	3	.90	1 egg yolk	16	48.0	14.5
Eggs, duck's, whole	4.0	1.2	1 egg	65	260.0	78.7
Milk, evaporated	.5	.15	½ cup	120	60.0	18.1
Milk, skimmed	.5	.15	1 cup	240	120.0	36.3
Milk, skimmed powder	3.0	.90	4 tablespoons	30	90.0	27.2
Milk, whole	.45	.13	1 cup	240	108.0	32.7
Milk, whole powder	2.5	.75	4 tablespoons	30	75.0	22.7
Fats ‖						
Butter	0	0	1 tablespoon	15	0	0
Crisco	0	0	1 tablespoon	15	0	0
Lard	0	0	1 tablespoon	15	0	0
Oil	0	0	1 tablespoon	15	0	0
Oleomargarine	0	0	1 tablespoon	15	0	0

* Thiamin values of food per gram are based on preferred values, Chapter XVIII.
† 3.3 γ thiamin = 1 I.U. See page 168 for the explanation of the International Unit.
‡ The measure and the weight of dry cereal are given. In cases where foods undergo great changes in weight during cooking, the approximate measure and weight are listed below in parentheses.
§ 2 tablespoons of flour is the approximate amount in 1 slice of bread or 1 biscuit.
‖ See page 277 regarding significance of fats.

TABLE V — *Continued*

Food	Thiamin * γ per Gm.	I.U. per Gm.	Approx. Size of Serving	Wt. of Serving in Gm.	Thiamin γ per Serving	I.U. Serving †
Fruits						
Fresh fruit, raw	.5	.15	1 average serving	120	60	18.1
Fresh fruit, cooked	.5	.15	1 average serving	120	60	18.1
Dried fruit, cooked	.5	.15	1 average serving	120	60	18.1
Dried fruit, raw	2.0	.60	1 average serving	30	60	18.1
Meat and Fish						
Group A — muscle tissue cooked, all except pork	.5	.14	1 average serving	90	45	13.6
Group B — pork muscle, cooked	3.0	.90	1 average serving	90	270	81.8
bacon, cooked	1.0	.30	2 average strips, fried crisp	20	20	6.0
Group C — organs, heart, cooked	2.0	.60	1 average serving	90	180	54.5
kidney, cooked	2.0	.60	1 average serving	90	180	54.5
liver, cooked	2.0	.60	1 average serving	90	180	54.5
Fish, fresh, unspecified, cooked	.4	.12	1 average serving	90	36	10.9
dried	.4	.12	1 average serving	90	36	10.9
Nuts						
Coconut, fresh	.3	.09	piece $2\frac{1}{2}''$ sq. $\frac{1}{4}''$ thick	50	15.0	4.5
Peanuts	8.0	2.4	30 medium size nuts	30	240.0	72.7
Other nuts	4.0	1.2	1 ounce	30	120.0	36.3
Sugar and Sirup						
Honey, strained	0	0	2 tablespoons	40	0	0
Honey, comb	0	0	2 tablespoons	40	0	0
Molasses, cane#	trace	trace	$\frac{1}{4}$ cup	71	trace	trace
Molasses, beet	trace	trace	$\frac{1}{4}$ cup	71	trace	trace
Molasses, sorghum	trace	trace	$\frac{1}{4}$ cup	71	trace	trace
Sugar, brown	trace	trace	$\frac{1}{4}$ cup	41	trace	trace
Sugar, white	0	0	$\frac{1}{4}$ cup	52	0	0

See page 234.

Food	Thiamin* γ per Gm.	I.U. per Gm.	Approx. Size of Serving	Wt. of Serving in Gm.	Thiamin γ per Serving	I.U. Serving†
Vegetables						
Group A — all vegetables except dried vegetables, potatoes, and peas, raw . . .	.5	.15	1 average serving	100	50	15.1
cooked	.3	.09	1 average serving	100	30	9.0
Group B — potatoes, sweet or white, cooked	.7	.21	1 medium size, or ¾ cup mashed	150	105.0	31.8
peas, fresh, green, cooked	2.0	.60	1 average serving	100	200.0	60.6
peas, canned	1.0	.30	1 average serving	100	100.0	30.3
Group C — legumes, dried, cooked° . . .	2.5	.75	½ cup	120	300.0	90.0

° Fresh beans, eaten with pods, should be classed under A.

TABLE VI

Summary of the Thiamin Value of a Few Therapeutic Diets

Type of Diet	Gms. P	Gms. F	Gms. C	Total Cal.	Thiamin γ	I.U.* Thiamin	T / Cal.	Non-Fat Calories	T / N.F.C.
Allergy Diet (only the following foods allowed: rice, lettuce, spinach, carrots, beets, artichokes, lamb, lemon, grapefruit, pears, sugar, Wesson Oil, Crisco, olive oil, gelatin, olives)	53	85	201	1781	507.0	153.6	0.284	1016	0.50
Diabetic Diet A, with whole wheat bread	60	70	100	1270	846.5	256.5	0.667	640	1.32
Diabetic Diet A, with white bread	60	70	100	1270	540.0	163.6	0.435	640	0.84
Diabetic Diet B, with whole wheat bread and whole grain cereal	70	85	130	1565	1000.5	303.1	0.638	800	1.25
Diabetic Diet B, with white bread and finely milled cereal	70	85	130	1565	633.0	191.8	0.404	800	0.79
Diabetic Diet C, with whole wheat bread and whole grain cereal	80	100	185	1960	1355.0	410.6	0.681	1060	1.28
Diabetic Diet C, with white bread and finely milled cereal	80	100	185	1960	812.0	246.0	0.414	1060	0.76
Ketogenic Diet (Fa/G 3.5)	58	295	18.5	2961	413.0	125.1	0.139	306	1.35
1000 Cal. Diet, well planned, with whole wheat bread	53	44	104	1024	900.0	272.7	0.879	628	1.43
1000 Cal. Diet, well planned, with white bread	53	44	104	1024	681.0	206.3	0.663	628	1.09
1000 Cal. Diet, poorly planned	41	38	135	1046	230.5	69.8	0.220	704	0.32
Desirable minimum	—	—	—	—	1000	300	0.500	—	1.00

* The International Unit has a rather indefinite significance (see p. 168), but it is used here because it is familiar to many physicians and dieticians.

TABLE VII

1000 Calorie Diet Well Balanced

	Food	Wt. in Gms.	Size of Serving	Gms. P.	Gms. F.	Gms. C.	Thiamin γ
Breakfast	Orange juice	120	½ cup	0	0	12	60
	Whole wheat bread	12	½ average slice	1	0	6	50
	Butter	2	½ level teaspoon	0	2	0	0
	Egg	50	1 egg	7	5	0	50
	Milk	200	1 average glass	7	8	10	90
	Coffee						
Lunch	Egg	50	1 egg	7	5	0	50
	5% vegetable, cooked	100	1 average serving	1	0	5	30
	5% vegetable, raw	100	1 average serving	1	0	5	50
	Whole wheat bread	25	1 average slice	2	0	12	100
	Butter	5	1 level teaspoon	0	4	0	0
	Fresh fruit	120	1 average serving	1	0	12	60
	Milk	200	1 average glass	7	8	10	90
Dinner	Lean meat, cooked	60	2 ounces	14	8	0	30
	5% vegetable, cooked	100	1 average serving	1	0	5	30
	5% vegetable, raw	100	1 average serving	1	0	5	50
	Whole wheat bread	25	1 average slice	2	0	12	100
	Butter	5	1 level teaspoon	0	4	0	0
	Fresh fruit	120	1 average serving	1	0	10	60
	Coffee						
				53	44	104	900 γ or 272.2 I.U

Calories from protein	212
Calories from fat	396
Calories from carbohydrate	416
Total calories	1024

Note: If whole wheat bread is replaced by white bread, the thiamin value of the diet will be 681, or 206.3 I.U.

TABLE VIII

1000 Calorie Diet Poorly Planned

	Food	Wt. in Gms.	Size of Serving	Gms. P.	Gms. F.	Gms. C.	Thiamin γ
Breakfast	Toast, white bread	50	2 average slices	4	0	24	25.0
	Cream	30	2 tablespoons	1	6	1	10.5
	Sugar	5	1 teaspoon	0	0	5	0
	Coffee						
Lunch	Clear broth	200	1 average bowl	0	0	0	0
	Melba toast (white bread, toasted)	50	2 average slices	4	0	24	25.0
	Grapefruit	120	1 average serving	1	0	12	60.0
	Sugar	5	1 teaspoon	0	0	5	0
	Tea						
Dinner	Lean meat or fish	90	3 ounces	21	12	0	45.0
	5% vegetable, cooked	100	1 average serving	1	0	5	30.0
	Baking-powder biscuit	75	2 medium size biscuit	5	6	31	21.0
	Butter	10	2 level teaspoons	0	8	0	0
	Cake (Angel cake)	37	piece $1\frac{1}{4} \times 2 \times 2\frac{1}{2}''$	3	0	22	3.5
	Cream	30	2 tablespoons	1	6	1	10.5
	Sugar	5	1 level teaspoon	0	0	5	0
	Coffee						
				41	38	135	230.5 γ or 69.8 I.U.

Calories from protein	164
Calories from fat	342
Calories from carbohydrate	540
Total calories	1046

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BIBLIOGRAPHY

1. Aykroyd, W. R.: Vitamins and Other Dietary Essentials. Heineman. London, 1936, p. 73.
2. Stiebeling, Hazel K.: Nutritive Value of Diets of Families of Wage Earners and Clerical Workers in North Atlantic Cities. U. S. Dept. of Labor, Serial No. R 409.
3. Diets of Families of Wage Earners and Low Salaried Clerical Workers Living in Industrial Communities in Three Regions of the United States. Bureau of Home Economics, U. S. Dept. of Agriculture R-6, May 27, 1937.
4. Baker, A. Z., Wright, M. D., and Drummond, J. C.: The Nutritive Value of Bread. Jour. Soc. Chem. Ind. *56*, 191 T, 1937.
5. Jansen, B. C. P.: On the Need of the Animal Organism of Antiberiberi Vitamin and on the Amount of This Vitamin in Various Foodstuffs. Mededeel. Burgerl. Geneesk. Dienst. Ned. Ind. 1923, pp. 1–118.
6. Rose, M. S., and Phipard, E. H. F.: Vitamin B and G Values of Peas and Lima Beans under Various Conditions. Jour. Nutrition *14*, 55, 1937.

GENERAL BIBLIOGRAPHY

AYKROYD, W. R.: Vitamins and Other Dietary Essentials. William Heinemann, Ltd., London, 1936.

BRADDON, W. L.: The Cause and Prevention of Beriberi. Rebman, Ltd., London, 1907.

COBB, S.: A Preface to Nervous Disease. Wm. Wood and Company, Baltimore, 1937.

COWGILL, G. R.: The Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.

EDDY, W. H., and DALLDORF, G.: The Avitaminoses. Williams and Wilkins Company, Baltimore, 1937.

FRASER, H., and STANTON, A. T.: Collected Papers on Beriberi: Studies from the Institute for Medical Research, Federated Malay States. John Bale, Sons and Danielsson, Ltd., London, 1924.

FUNK, C.: the Vitamins. Translated by H. E. Dubin. Williams and Wilkins Company, Baltimore, 1922.

HARRIS, L. J.: Vitamins in Theory and Practice. The Macmillan Company, New York, 1935.

MCCARRISON, R.: Studies in Deficiency Disease. Oxford Medical Publications. Henry Frowde and Hodder and Stoughton, London, 1921.

MCCOLLUM, E. V., and SIMMONDS, N.: The Newer Knowledge of Nutrition, 4th Ed. The Macmillan Company, New York, 1929.

MCLESTER, J. S.: Nutrition and Diet in Health and Disease, 2d Ed. W. B. Saunders Company, Philadelphia, 1934.

MATHEWS, A. P.: Vitamins, Minerals and Hormones. William Wood and Company, Baltimore, 1937

MELLANBY, E.: Nutrition and Disease. Oliver and Boyd, London, 1934.

ROSE, M. S.: The Foundations of Nutrition, Revised Ed. The Macmillan Company, New York, 1933.

SHERMAN, H. C.: Chemistry of Food and Nutrition, 5th Ed. The Macmillan Company, New York, 1937.

SHERMAN, H. C., and SMITH, S. L.: The Vitamins, 2d Ed. The Chemical Catalog Company, Inc., New York, 1931.

STITT, E. R.: The Diagnostics and Treatment of Tropical Diseases, 5th Ed. P. Blakiston's Son and Company, Philadelphia, 1929.

SURE, BARNETT: The Little Things in Life. D. Appleton-Century Company, New York, 1937.

VEDDER, E. B.: Beriberi. William Wood and Company, New York, 1913.

VEDDER, E. B.: Beriberi and Epidemic Dropsy. Tice's Practice of Medicine, Vol. IV. W. F. Prior Company, Hagerstown, Maryland, 1936.

WENCKEBACH, K. F.: Das Beriberi-Herz. Julius Springer, Berlin und Wien, 1934.

PART II

HISTORICAL AND EXPERIMENTAL

CHAPTER IX

DISCOVERY OF THE VITAMINS

During the era of exploration inaugurated by Columbus, the lure of the gold and gems of India and Cathay was held forth as the incentive alike to monarchs and sailors. However, a retrospective survey of this era and of the subsequent colonial expansion of Western nations reveals small returns in gold to Europe. Indeed, when one takes into account not only commerce, but also the expense of outfitting armies, as well as the humanitarian enterprises of the West, as expressed in missionary ventures of the church and of modern medical foundations, the flow of wealth has probably been predominantly in the opposite direction. Such material benefits as have accrued have been mutual and have resulted from exchange of products and ideas.

The exchange has not always been beneficial, for we have traded infectious diseases with the East and there is nowadays a growing clash of alien social and national traditions. The art of medicine, though not necessarily the state of public health, has benefited from the exchange. Europe got the plague from the Orient in the late Middle Ages, but certain aspects of its appearance remained a puzzle till the outbreak of pneumonic plague in Manchuria in 1912. Then an International Commission determined the organism to be identical with that of bubonic plague and so cleared up discrepancies in the stories of the Black Death of London in 1348. We got cinchona bark from "Indians" of South America, ephedrine from China, and chaulmoogra oil from Burma as samples of primitive medicine. In exchange we gave a choice selection of our maladies and, during the present generation, a bonus of some substantial aids of modern medicine.

The contact between East and West has played an important part in opening up new chapters of medicine, among them that of the deficiency disorders, a chapter which may some day be regarded as rivaling in importance the discovery of microorganisms as causative factors in disease. The contributions of the Japanese in their own country, of the Dutch colonizers in the East Indies, of British officials in India and the Malay

States, and of American physicians in the Philippines not only immeasurably forwarded the study of beriberi directly but had much to do with inspiring dietary researches in Europe and America. Independent nutritional studies in the West might have effected an unraveling of the tangled clues to these disorders without intervention of the East, but it is highly improbable that general interest should have been aroused so early nor have been so well sustained without the picture before us of a dramatic disease of dietary origin affecting the teeming millions of Oriental lands. One would not have predicted that Western nations might ultimately profit more by study of its poverty and distress than by seizure of the wealth of the fabled East. No more can one predict that the names of Takaki, Eijkman, Funk, and perhaps others may not be held in an esteem by a future generation comparable with the reverence which we accord to Pasteur and to Lister.

Vitamin B_1, as the authors prefer to designate it for reasons which will later appear, first came to light through the activities of students of the Oriental disease, beriberi. One of the earliest advocates of the dietary theory of the origin of beriberi was the Surgeon General of the Japanese Navy, Takaki (1). He became convinced of this idea by observing the disease in the Japanese Navy and was able in 1884, by change in the ration, to eradicate it almost completely. Whereas between the years 1878 and 1883, inclusive, there had been an incidence of the disease ranging from 23 to 40 per cent of the entire force, the incidence has not reached even ½ per cent in any year since 1885. The principal change in the diet which was responsible for this astonishing result was the substitution of a moderate amount of meat and legumes for a portion of the rice which had previously constituted a substantial fraction of the food. Later, partial substitution of barley for rice was also practiced. The dietary changes were sufficient in number to render difficult an adequate analysis of their precise significance. It was Takaki's early opinion that the improvment resulted from an increase in the protein content of the food.

Takaki's experience converted few students of the disease to the dietary theory, and a more specific hypothesis as to the cause of beriberi did not develop until 1897, when Eijkman (2) in Java demonstrated that a paralytic condition resembling beriberi could be developed in fowls by feeding them upon an exclusive diet of polished rice. As so often happens, this important discovery was due at the outset to accident, a few cases of avian beriberi developing spontaneously among chickens which were cooped up at the laboratory, in the course of studies intended to demonstrate the infectious character of the disease. In an extended series of studies, Eijkman demonstrated that

the disease did not develop if the fowls were fed upon unpolished rice, and that it could be prevented by the administration of rice polishings to birds which were otherwise fed ad libitum upon polished rice.

Eijkman recognized the resemblance of the experimental disease produced in fowls to that of beriberi and after a few years came to believe, with his successor Grijns, that the diseased condition was due to the deficiency of some substance which was present in abundance in the whole rice grain, but absent from the starchy kernel. In this way the deficiency theory of the origin of beriberi came to be definitely formulated. This theory, however, had few adherents for many years, and was opposed by those who attributed beriberi to some form of intoxication or infection. It was not until Fletcher at Kuala Lumpur in 1905 (3) and Fraser and Stanton in 1909–1910 published their important studies of the human disease in the Malay States that this idea regarding its etiology was taken seriously outside the Dutch colonies. Fletcher's experiment was conducted in an insane asylum which had been subject periodically to epidemics of beriberi. At the outset the patients were all numbered, the odd-numbered ones going to one ward, the even to another. The first ward was supplied with white rice, the second with brown (cured) rice. Of 153 patients who occupied the first ward for longer than a month 65 developed beriberi in the course of the experiment, which was of two years' duration; of 154 patients who remained for similar times in the second ward not one developed the disease.

The other notable research involved three hundred laborers divided between two railroad labor camps. To one of these Fraser and Stanton issued the customary white rice as a staple article of diet; to the other, "cured" rice, retaining a large part of the bran coats. In about three months' time a severe epidemic of beriberi occurred in the white-rice camp, while the cured-rice camp remained free from the disease. Later the rice issues were reversed in the two camps. This was promptly followed by a sharp reduction and disappearance of the disease in the first camp and the subsequent development of an epidemic of beriberi in the second. These experiments, together with many others which presently followed, carried conviction of the essential soundness of Eijkman's deficiency theory as accounting for the human disease. An excellent account of this Oriental history is contained in Vedder's book (5). This publication was very influential among medical circles in the East because of its careful analysis of the evidence and because Vedder himself had been a moving spirit in eradicating beriberi from the Philippine Scouts by introducing unpolished rice into their ration.

The book, however, which attracted world-wide attention

was that of Funk (6). Apparently Funk caught much of his inspiration from Fraser and Stanton, for his early chemical experiments followed along the lines which the latter had pursued. Funk, however, after what must have been a wide and intensive course of reading of the literature, boldly projected the deficiency theory as the cause not only of beriberi but also of scurvy, rickets, and pellagra. The scientific intuition displayed in his book was of the highest order, and the much maligned name, vitamin(e), which he gave to the exogenous hormones which he supposed necessary for the prevention of these disease conditions was a stroke of genius. It is unfortunately true that the word has been capitalized by charlatans to prey upon the gullibility of doting mothers in an unconscionable way. However, such a captivating word was necessary to focus the attention of the public and, in turn, of science upon the possibilities of the field. Vedder's more staid "beriberi preventing substance," Susuzki's term "oryzanin," or Edie's word "toruline" not only lacked dramatic value but displayed a lesser vision or a lesser courage. In the word itself Funk revealed that he conceived the substance as something of significance beyond the Orient and pervading living things generally, not merely rice (*Oryza sativa*) or yeast (*torula*). Such guessing of future developments is thoroughly detrimental if the guess is wrong, but when it proves right, science leaps forward. Very likely it was at least partly Pasteur's uncanny capacity to discern the answer in advance that incurred so much antagonism among his contemporaries.

But as Pasteur and Lister had their Jenner, who had fifty years before demonstrated a special case of what they later conceived as of general significance, so Eijkman and Funk had forerunners who did not succeed in making their voices heard. Lunin at Basle in 1881 had postulated that "a natural substance such as milk must therefore contain, besides these known principles, small quantities of unknown substances essential to life." Pekelharing, countryman of Eijkman, was responsible for his appointment for the special study of beriberi in Java, but strongly encouraged him to seek an infectious cause (7). Eijkman's findings so altered the course of his studies and his thought that he said in 1905 "there is a still unknown substance in milk which even in very small quantities is of paramount importance in nutrition. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and, with apparent abundance, the animals die of want. Undoubtedly this occurs not only in milk but in all sorts of foodstuffs, both of vegetable and animal origin" (8).

By this time the idea was in the air, for Hopkins in England declared his belief in 1906 in "accessory food factors" in milk.

Holst and Frölich were already engaged in a study of ship-beriberi which seriously affected Norwegian sailors during long cruises (see page 24). Following Eijkman's lead, they used polished rice as a diet for their experimental animals but chose to substitute guinea pigs for fowls, believing that mammals would give results of greater significance for human nutrition. Because of the guinea pig's sensitivity to lack of what we now call vitamin C, it was experimental scurvy rather than beriberi which developed first. Scurvy had, of course, long been known, and lime juice was to some extent advocated as a specific protection against it. However, scurvy and ship-beriberi had often appeared together and by some were thought to be identical diseases, undoubtedly for the reason that sailors had not infrequently suffered from both diseases at once. Nevertheless the confusion between the two for the time being only added to the obscurity and raised the question whether a single deficiency could cause two different diseases.

In the meantime other very significant studies of animal nutrition were in progress in Europe and the United States. Stepp (9) observed that mice lived satisfactorily upon a diet of bread which was made with milk, but that a decline in weight and death soon ensued if the bread was previously extracted with alcohol. The damage to the bread, as a foodstuff, could be repaired by restoring to it the substances contained in the alcohol extract. Similarly, Hopkins (10) found that rats could not long survive on a diet of purified protein, carbohydrates, fats, and mineral salts, but that the addition of milk in sufficient quantity to supply about 4 per cent of the total dry weight of the food mixture resulted in satisfactory nutrition.

During the same period that the above experiments were in progress McCollum in the United States was beginning an analysis of the dietary requirements of the rat. He and his associates, by the simple expedient of adding a small proportion of butter fat to the diet, secured growth in young rats fed upon an otherwise inadequate mixture of foodstuffs which they regarded as "purified." These workers became convinced that "with foodstuffs of known character there was but a single unidentified substance necessary in the diet," that is, the substance supplied by butter (11). This was directly at variance with the views of the beriberi school, for butter does not contain the beriberi-preventing vitamin which they were inclined to think of as the only substance worthy of the name. However, presently McCollum and Davis in the course of comparing a series of somewhat similar diets discovered that certain supposedly pure carbohydrates were not actually the nutritional equivalent of other supposedly equally pure substances of this character. More specifically it was found that lactose which they had used

in their artificial diet could not be replaced by starch. Further, by repeated recrystallization the original lactose could be deprived of its growth-promoting property (12). As we now know, the lactose contained a small amount of vitamin B_1 which, as this experience showed, was for a time confused with the wholly dissimilar oil-soluble vitamin of butter fat. Butter fat and cod liver oil, as later experimentation has shown, supplied the necessary vitamin A (and D), but not the equally essential B.

Once convinced of the necessity of recognizing a multiplicity of vitamins, science began to make better progress. In spite of the reluctance of the graybeards to have young upstarts postulate new substances, the number of recognized vitamins has continued to grow steadily in the intervening years. Bit by bit they have been distinguished by differences in their occurrence, their stabilities, and the pathologies due to their deficiencies. Many of them have since been isolated as pure substances, their chemical structure has been more or less fully ascertained, and the artificial synthesis of some has been accomplished. Chemically speaking, they constitute an utterly diverse category of substances which share nothing in common except a definition which is stated in physiological terms: "specific organic substances occurring in foodstuffs in small quantities and essential to the life of animals." In view of their chemical diversity, no general statements can be made about their chemical or physical properties. That they have some connecting thread of similarity as to physiological function seems very likely from certain kinships of the pathological changes which are induced by a deficiency of them. But one cannot say that vitamins are destroyed by heat, that they are found in fresh foods, or that they are produced by sunlight. Such statements are true only of single individuals or of purely artificial groupings of them. Some are a thousand times more potent than others, yet all are equally indispensable for normal functions and are in no sense interchangeable in physiological utility.

BIBLIOGRAPHY

1. Takaki, K.: Three Lectures on the Preservation of Health amongst the Personnel of the Japanese Navy and Army. Lancet (1906) I, 1369, 1451, 1520.
2. Eijkman, C.: Eine beriberiähnliche Krankheit der Hühner. Arch. Path. Anat. (Virchow's) *148*, 523, 1897.
3. Fletcher, W.: Rice and Beriberi. Jour. Trop. Med. and Hyg. *12*, 127, 1909.

4. Fraser, H., and Stanton, A. T.: An Enquiry Concerning the Etiology of Beriberi. Lancet (1909) I, 451. White Rice as a Causative Agent in Beriberi. Lancet (1909) II, 406. The Etiology of Beriberi. Studies from Institute of Medical Research, Fed. Malay States, No. 10, 1909; No. 12, 1911.
5. Vedder, E. B.: Beriberi. New York, Wm. Wood and Company, Baltimore, 1913.
6. Funk, C.: Die Vitamine. J. F. Bergmann, Wiesbaden, 1914.
7. Jansen, B. C. P.: Über Aneurin (=Vitamin B_1). Klin. Wochschr. *16,* 113, 1937.
8. Van Leersum, E. C.: The Discovery of Vitamins. Science *64,* 357, 1926.
9. Stepp, W.: Versuche über Fütterung mit lipoidfreier Ernährung, Biochem. Zeitschr. *22,* 452, 1909.
10. Hopkins, F. G.: Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries. Jour. Physiol. *44,* 425, 1912.
11. McCollum, E. V.: Newer Knowledge of Nutrition, 1st Ed. The Macmillan Company, New York, 1919, p. 27.
12. McCollum, E. V., and Davis, M.: The Essential Factors in the Diet during Growth. Jour. Biol. Chem. *23,* 231, 1915.

CHAPTER X

DIFFERENTIATION OF VITAMIN B

By 1920 the beriberi vitamin was no longer in danger of being confused with the antirachitic or the fat-soluble ones. However, another source of perplexity existed which is by no means fully dissipated at the present time, although a vast progress has been made. Mitchell (1) was the first to question seriously whether the substance detected in yeast, for example, by the rat growth test was actually identical with the antineuritic factor which prevented or cured polyneuritis in birds. He called attention to numerous apparent disparities of the results of testing foodstuffs by the two methods. Notably the green vegetables, roots, and tubers had a far more potent influence on the growth of young rats than could be accounted for on the basis of their prophylactic value against polyneuritis. Such evidence had the weakness that foodstuffs could well be expected to vary in vitamin content according to maturity or state of preservation, and Mitchell's question did not immediately receive the attention it deserved. Certainly the similarity in solubility of the "water-soluble B" from various sources was such as to encourage belief in the identity of the two.

The stability of "water-soluble B" to heat was soon made the subject of a study which indicated greater lability of the antineuritic potency than of the growth-promoting quality (2). However, apparently valid counter-arguments were advanced, no one conceiving the problem with sufficient clarity to plan a rigorous experiment. The question was too generally framed in terms of identity or non-identity of the antineuritic and growth factors, little thought being given to the possibility of a multiplicity of substances present generally in foodstuffs but in varying proportions. Eijkmann (3) and Jansen (4) were among the early skeptics and declared against the rat-growth test for determining the antineuritic vitamin.

Eventually quite definite evidence was supplied by Smith and Hendrick (5). These experimenters demonstrated that yeast which had been autoclaved for six hours at fifteen pounds' pressure still retained the property of promoting growth in rats on certain diets which were otherwise inadequate. Since it had long ago been demonstrated that the vitamin of rice polish and

other brans was destroyed by such a treatment, it was evident that yeast contained a thermostable factor in addition to the labile one which had long been recognized as being present. Both are required for the growth of rats, but the need of adult birds for the thermostable factor is so slight that it cannot be detected with certainty. Thus came into being a knowledge of the second of the now numerous family of B vitamins.

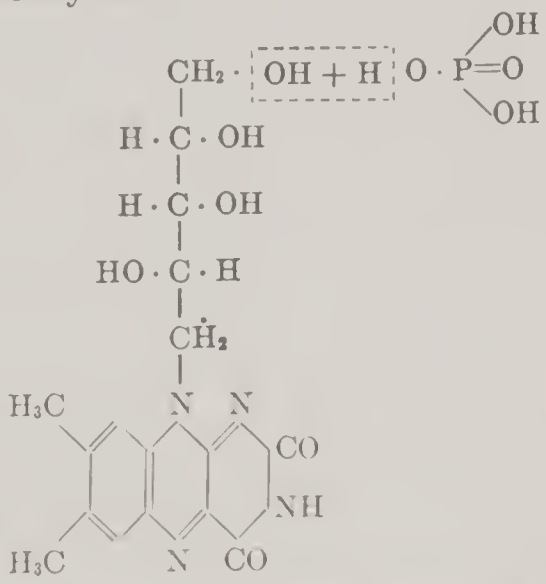
While all this was in progress, Goldberger and associates were studying human pellagra and had become convinced that some of the foodstuffs that promote rat growth and prevent polyneuritis also prevent pellagra (6). Yeast, milk, and beef were specified as materials rich in the pellagra-preventing (P-P) factor. They were also shown to be effective in experimental black tongue in dogs (7), a disease closely resembling pellagra.

Inasmuch as it had been discovered that rats on certain restricted diets developed a condition that was pellagra-like with respect to a dermatitis and ulceration or inflammation of the mouth (8), there was a great temptation to assume that the thermostable substance of Smith and Hendrick was the pellagra vitamin. Encouragement in this idea was supplied by evidence that rat dermatitis responded to administration of autoclaved yeast. Attempts were made in a score or more of laboratories to isolate this supposedly single thermostable factor which had in the meantime been blessed with new names, vitamin B_2 in England and vitamin G in America. As these endeavors progressed, a diversity of properties appeared, depending on the raw materials, the fractionation methods, and the species of animals chosen for test purposes. Gradually it was recognized that there are several forms of nutritional dermatitis in common laboratory animals and that, at least for the most part, they have no relation to the dermatitis of human pellagra. Gradually many biochemists became convinced that the search for *the* pellagra factor would have to be prefaced by the isolation and characterization of some of the components of the mixture. They severally addressed themselves to the study of that particular factor which each felt he could recognize with greatest certainty without regard to evidence of its importance in human nutrition.

In this way three factors of the original thermostable vitamin B complex have been definitely isolated, and the constitution of two of them has become known. In the order of their isolation as pure chemical compounds, they are: riboflavin (also called lactoflavin, vitamin B_2, and vitamin G), nicotinic acid, which is proving an important factor in human pellagra, and vitamin B_6, also called Factor 1. After discussing these we shall take up more briefly the less well defined components of the B complex.

Riboflavin was isolated from milk in pure crystals melting at 293° in 1933 (9). However, it was presently shown that although this substance, with proper supplements, helps to promote the growth of rats and prevents their developing nutritional cataract, it does not prevent rat dermatitis, affect the course of black tongue in dogs, or check the development of dermatitis in chicks. It is, however, a preventive for nutritional dermatitis in turkeys.

The chemistry of riboflavin was worked out in a beautiful way independently by Kuhn and by Karrer in 1935 and is well reviewed by Kuhn (10). It has the structure shown below. Phosphorylation of it at the indicated hydroxyl forms an ester which constitutes the prosthetic group of a yellow respiratory enzyme. The enzyme itself consists of this prosthetic group in combination with a protein. The two components can be separated by dialysis. Separately they have no activity; combined again, the physiological activity is immediately restored. The combined substance functions as a dehydrogenase. This fact appears highly suggestive with regard to other vitamins, and there are already indications that the antineuritic vitamin also functions as a coenzyme.

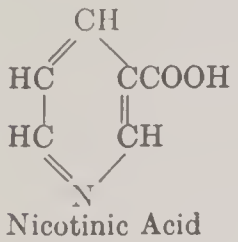


6,7-Dimethyl-9-(d-1′-ribityl)-isoalloxazine. Lactoflavin

As soon as this substance became available, attention was concentrated more fully on the remaining factors required for rat growth. The eluate of a charcoal which had been treated with yeast extract provided the missing substance(s) (11). Another preparation which has been much used is the eluate from fuller's earth, which had been treated with liver or rice polish extract. From such preparations there has recently been isolated in crystalline form the substance known as B_6 m.p. 204°–206° (12, 13). It cures and prevents rat dermatitis. However, even in combination with the antineuritic vitamin, riboflavin and

nicotinic acid, it cannot fulfill the entire function of the vitamin B complex, for the rats do not grow normally, though growth is improved by this addition.

To conclude the category of B vitamins which are at present known in pure form, we must discuss nicotinic acid. This substance was isolated from rice polish and yeast by Funk in 1911 in the course of his early studies of the beriberi vitamin. It has very recently been isolated as the amide from liver and found curative for black tongue in dogs (14). It is also, at least to an astounding degree, curative for human pellagra (15). It does not appear to affect the growth of rats.



Nicotinic Acid

The remaining components of the B complex are less well defined and cannot be discussed in detail. The process of differentiation of the B complex into its components is indicated diagrammatically in the Frontispiece to provide the reader at a glance with some conception of the complexity of the problem and the nature of the procedures employed. We have listed below the several factors which have been postulated and partially characterized by various workers. It will be recognized that the existence of some of them is disputed; that our classification of them according to adsorbability may be faulty; that we may have excluded some active principles that ought to be included or vice versa; and that in some cases one factor may be proved identical with another of at present different designation. In preparing the list we make no pretense of passing upon the validity of the evidence in the case of the incompletely identified components. Only specialists can do so, and even they do not fully agree. A few references to recent papers are included, which give an idea of the present status of the less fully characterized principles. Elvehjem has provided us with a recent review of the vitamin B complex (16).

Thermolabile Factors

1. Vitamin B_1, thiamin or aneurin
2. Vitamin B_3, necessary in addition to 1 for the weight maintenance of pigeons (17)
3. Vitamin B_4, regarded as necessary for growth of rats and to protect them against a paralysis (18)
4. W factor, the alcohol ether precipitate from liver extract. Protein in nature (19)
5. Antigizzard erosion factor, perhaps identical with or related to B_3 (20)

Thermostable Factors. Adsorbed (21)

1. Riboflavin, rat growth promoting, cataract preventing, turkey dermatitis preventing

2. Nicotinic acid, black tongue and pellagra curative (perhaps not the sole pellagra vitamin)
3. B_5, ill-defined thermostable pigeon weight factor (22)
4. B_6, rat growth promoting, antiacrodynia factor, isolated but not identified. Also called Factor 1.

Unabsorbed (Filtrate Factors or Factor 2) (24)

1. Chick dermatitis factor (25)
2. Gray hair preventing factor (26)

Unclassified Factors Possibly Associated with the B Complex

1. Extrinsic factor (27)
2. Anti egg white injury factor (28, 29)
3. Antianemic factor (30)
4. Choline (31)

It is very evident that in such a bewildering state of affairs the matter of nomenclature is quite important. The terms vitamin B_2 and vitamin G are still unfortunately used in several senses, the most popular being (1) as synonymous with riboflavin and (2) to embrace the entire thermostable B component in foods. In the latter sense there is, of course, no such thing as vitamin G, and assays of foodstuffs made on this basis have no ascertainable significance. Assays of foodstuffs for their riboflavin content are, however, quite significant. Unfortunately, one often cannot tell from the context in what sense these and similar terms are being used by the authors. This is particularly true of articles in abstract form.

In America, the beriberi or antineuritic vitamin is often referred to as vitamin B. This term is somewhat objectionable because it has been used more or less in the past to include the whole category of substances of physiological importance which have been associated together in experimental studies. We prefer the term vitamin B_1 which originated in England and has long prevailed in Europe. The subscript numeral recognizes that it is one of the group of substances belonging to the B classification and serves to emphasize the fact that we are in the midst of a process of resolving a complex.

Since vitamin B_1 is now fully identified, there is much reason to give it a more convenient name. Professor Jansen who first isolated the substance has suggested the name aneurin (32). His achievement clearly entitles him to the honor of designating it, but the name which he has selected has been objected to by the American Medical Association as carrying an implication of therapeutic utility. The name thiamin has been suggested to connote the idea that it is the sulfur containing vitamin (33). It has some advantage over aneurin in that it does not imply a specific connection with nerve function. This conforms to a large

amount of evidence that this vitamin has a function even in organisms which have no nervous systems.

This was done after correspondence with Professor Jansen which at the time seemed to indicate his concurrence. Later, after consulting various of his European colleagues, he suggested that the matter be referred to the International Committee on Nomenclature, but the American Society of Biological Chemists, the American Institute of Nutrition, and the Committee on Nomenclature of the American Chemical Society had tentatively approved the name thiamin. The Council of the American Medical Association has adopted it with the proviso that if the International Committee in 1938 should adopt some other suitable name, the Council will concur in its use, with thiamin as a synonym. Early availability of the product to the American physician was the only consideration prompting the original proposal of this name, as far as the present authors are concerned.

The reader should not feel discouraged if he finds the nomenclature and other intricacies of the B complex confusing. A large number of laboratories have participated in the study, and very often papers have appeared simultaneously from two or more of them, each relating new discoveries and each coining new terms to describe them. Moreover, the observations of each have usually concerned themselves with different aspects of the matter in hand. Consequently one author has often been quite uncertain as to whether or not the factor he has observed is or is not identical with that observed by another. No two specialists in the field can agree fully on the identities or even specific properties of all the factors. It should be said, however, that the identification of four members of the complex will greatly hasten further resolution. Each of the four known substances can now be added to experimental diets in pure form and thus greatly reduce the number of unintentional variables. In the meantime one must read the literature carefully to ascertain from the context, or otherwise, the sense in which the various names are used by a particular author.

BIBLIOGRAPHY

1. Mitchell, H. H.: On the Identity of the Water Soluble, Growth Promoting Vitamin and the Antineuritic Vitamin. Jour. Biol. Chem. *40,* 339, 1919.
2. Emmett, A. D., and Luros, G. O.: Are the Antineuritic and Growth Promoting Water Soluble Vitamins the Same? Jour. Biol. Chem. *43,* 265, 1920.

3. Eijkman, C., Hoogenhuijze, C. J., and Derks, T. J. G.: The Vitamin Content of Microorganisms in Relation to the Composition of the Culture Medium. Jour. Biol. Chem. *50*, 311, 1922.
4. Jansen, B. C. P.: On the Need of Antiberiberi Vitamin of the Animal Organism and on the Amount of This Vitamin in Different Foodstuffs. Mededeel. Burgerl. Geneesk. Dienst. Ned. Indie. *I*, 1, 1923.
5. Smith, M. I., and Hendrick, E. G.: Some Nutrition Experiments with Brewers' Yeast. U. S. Public Health Reports, *41*, 201, 1926.
6. Goldberger, J., and Tanner, W. F.: A Study of the Pellagra Preventive Action of Dried Beans, Casein, Dried Milk and Brewers' Yeast with a Consideration of the Essential Factors Involved. U. S. Public Health Reports, *40*, 54, 1925.
7. Goldberger, J., and Wheeler, G. A.: Experimental Blacktongue of Dogs and Its Relation to Pellagra. U. S. Public Health Reports, *43*, 172, 1928.
8. Goldberger, J., and Lillie, R. D.: A Note on an Experimental Pellagra-like Condition in the Albino Rat. U. S. Public Health Reports, *41*, 1025, 1926.
9. Kuhn, R., György, P., and Wagner-Jauregg, Th.: Über eine neue Klasse von Naturfarbstoffen. Vorlaufige Mitteilung. Ber. deut. chem. Ges. *66*, 315, 1933. Über Ovoflavin, den Farbstoff des Eiklars. *Ibid.*, p. 576. Über Lactoflavin, den Farbstoff der Molke, p. 1034. Über die aus Eiklar und Milch isolierten. Flavine, p. 1577. Kuhn, R., Rudy, H., and Wagner-Jauregg, Th. Über Lactoflavin (Vitamin B_2). Ber. deut. chem. Ges. *66*, 1950, 1933.
10. Kuhn, R.: Über die Flavine. Z. angew. Chem. *49*, 6, 1936. See also H. Tauber, Enzyme Chemistry. John Wiley & Sons, New York, 1937, p. 203.
11. Gyorgy, P.: Differentiation of Lactoflavin and the Rat Antipellagra Factor. Biochem. J. *29*, 741, 1935.
12. Keresztesy, J. C., and Stevens, J. R.: Crystalline Vitamin B_6. Proc. Soc. Exp. Biol. Med. *38*, 64, 1938.
13. Lepkovsky, S.: Crystalline Factor 1. Science *87*, 169, 1938.
14. Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W.: Isolation and Identification of the Anti-Black Tongue Factor. Jour. Biol. Chem. *123*, 127, 1938.
15. Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H.: Treatment of Human Pellagra with Nicotinic Acid. Proc. Soc. Exp. Biol. Med. *37*, 405, 1937.
Spies, T. D., Cooper, C., and Blankenhorn, M. A.: Use of Nicotinic Acid in the Treatment of Pellagra. J.A.M.A. *110*, 622, 1938.
16. Elvehjem, C. A.: The B Vitamins except B_1 and the Flavins. Ergebnisse d. Vitamin u. Hormonforsch. *1*, 140, 1938.
17. Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon. The Effect of Vitamin B_1. Biochem. J. *31*, 2264, 1937.

18. Kline, O. L., Elvehjem, C. A., and Hart, E. B.: Further Evidence for the Existence of Vitamin B_4. Biochem. J. *30,* 780, 1936.
19. Frost, D. V., and Elvehjem, C. A.: Further Studies on Factor W. Jour. Biol. Chem. *121,* 255, 1937.
20. Bird, H. R., Kline, O. L., Elvehjem, C. A., Hart, E. B., and Haplin, J. G.: Distribution and Properties of the Anti-gizzard Erosion Factor Required by Chicks. Jour. Nutrition *12,* 571, 1936.
21. Schultz, H. W., and Mattill, H. A.: Studies on the Vitamin B Complex. Jour. Biol. Chem. *122,* 183, 1937.
22. Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon. Vitamin B_5. Biochem. J. *31,* 2270, 1937.
23. Euler, H. v., Malmberg, M., Schlenk, F., and Gleim, W.: Vitamin Studies II. Ark. Kemi Mineral. Geol. *12B,* No. 33, 1937.
24. Lepkovsky, S., Jukes, T. H., and Krause, M. E.: Multiple Nature of the Third Factor of the Vitamin B Complex. Jour. Biol. Chem. *115,* 557, 1936.
25. Elvehjem, C. A., and Koehn, C. J., Jr.: Vitamin B_2 (G). The Non-identity of Vitamin B_2 and Flavins. Jour. Biol. Chem. *108,* 709, 1935.
26. Morgan, A. F., Cook, B. B., and Davison, H. G.: Vitamin B_2 Deficiencies as Affected by Dietary Carbohydrate. Jour. Nutrition *15,* 27, 1938.
27. Miller, F. R., and Pritchard, W. H. Presence in Milk of the Extrinsic Factor of Castle. Proc. Soc. Exp. Biol. Med. *37,* 149, 1937.
28. György, P.: Attempts to Isolate the Anti-egg Injury Factor (Vitamin H). Proc. Amer. Soc. Biol. Chem. VIII, 1937.
29. Parsons, H. T., Lease, J. G., and Kelly, E.: Interrelation between Dietary Egg White and the Requirement for a Protective Factor in the Cure of the Nutritive Disorder Due to Egg White. Biochem. J. *31,* 424, 927.
30. Hogan, A. G., Richardson, L. R., and Johnson, P. E.: A New Deficiency Disease Associated with the Vitamin B Complex. Proc. Amer. Soc. Biol. Chem. *L,* 1937.
31. McHenry, E. W.: The Effect of Choline upon the Weights of Young Rats. Jour. Physiol. *85,* 343, 1935.
32. Jansen, B. C. P.: Identity of Vitamin B_2 and Flavins and the Nomenclature of the Vitamins. Nature *140,* 267, 1935.
33. Editorial: Thiamin Chloride (Vitamin B_1). J.A.M.A. *109,* 952, 1937.

CHAPTER XI

ISOLATION OF THIAMIN

It is doubtful whether the isolation and identification of any other substance in the history of biochemistry have cost as much labor as have these operations as applied to thiamin. The first gram of the pure vitamin must have cost an aggregate of several hundred thousand dollars. To mention all the names of those who have participated in some phase of the project is to call the roll of half the mature biochemists in England and the United States. The project has bulked equally large upon the horizon of Dutch, Japanese, and French biochemistry during the twentieth century. German participation has been relatively small until within the current decade.

The most important reasons for the large outlay were: the highly multiple character of what once seemed a single substance; the meager concentration in which it occurs in natural sources; the slow and laborious character of bioassay methods; the multiplicity of other substances which are akin to it in solubilities; and the lability of the molecule toward chemical processing.

However, now that the task is done, the story is scarcely worth recounting in detail, so numerous, diffuse, and ill planned do many of the earlier efforts appear in the light of subsequent events. It will be well merely to sketch the activities of the principal groups which have participated and the character of the methods which they used. We must begin with Eijkman and Grijns, who by 1906 had demonstrated the solubility of the vitamin in water and in aqueous alcohol, its thermolability, and the fact that it readily diffuses through a membrane, thus proving the relatively small size of the molecule. Next should be mentioned Fraser and Stanton, who confirmed these findings, introduced the use of alkaloidal reagents, notably mercuric chloride, and observed increased lability of the substance in alkaline solution (1).

At about this time there were enlistments in France, England, Japan, and the Philippines. Funk first at Pasteur Institute, later at Lister, first enunciated the idea that the beriberi-preventing substance is a nitrogenous base and gave it the name vitamine. His use of phosphotungstic acid and of silver nitrate for precipita-

tion of the substance is historically important (2). The first use of an adsorbent in the form of an animal charcoal is believed to have been by Chamberlain and Vedder (3). Very shortly followed the contributions of groups of English (4) and Japanese (5) chemists, who experimented with a variety of reagents including lead acetate. Most of these have not proved very serviceable. The latter, however, records the first recognition of nicotinic acid as a component of rice polish, a fact which is of great interest in view of the action of this substance in curing black tongue as disclosed in 1937 (see page 133).

The next highly significant event in connection with isolation was the introduction of fuller's earth as an adsorbent by Seidell (6). This reagent or its equivalent has been of very great importance, not only for the isolation of thiamin but for the differentiation and isolation of other B factors.

There followed a rather long period, not of inaction but of relative futility, partly due to the interruption of the World War. Perhaps the somewhat bizarre efforts of Williams to approach the matter by synthesis (7) may be mentioned as a premonition of the recently developed importance of pyridine derivatives in biological agencies (see page 162). The beginning of German participation (8) and the entrance into the field of R. A. Peters (9), often in association with H. W. Kinnersley, belong to this period. Peters' persistent adherence to the use of charcoal rather than fuller's earth as an adsorbent served for a decade to illuminate the field from a different angle.

The first genuine success in isolating the substance came in 1926 in the same laboratory in Java where Eijkman had done his pioneer work thirty years previously. The now classical papers of Jansen and Donath stand out not only as a landmark in vitamin history but as an example of a systematically planned and executed pursuit of a chemical objective (10). The chemical methods represented refinements of those of Funk and Seidell and others making use of adsorption on acid clay, elution with baryta, and fractional precipitation with silver. *p*H control during the latter operation was an important feature. Success was, however, probably due as much to the selection of a good method of bioassay to gauge the progress of the chemical fractionation. Rice birds on a polished rice diet were used for this purpose. These small birds have the advantage that they contract the disease in ten to fourteen days. These were used in groups of ten to minimize the effects of individual variation. The vitamin was isolated as the hydrochloride, picrolonate, and gold salt.

No one was able, in spite of their clear directions, to reproduce the results of the Dutch experimenters during the succeeding six or seven years, so delicate was the procedure and so narrow the margin between success and failure. Ohdake obtained a crystal-

line product (11) by a similar method in 1932, but it appeared far less potent than that of the Dutch workers. However, the melting point, 250° C., was in agreement, and the substance was stated to be the hydrochloride of a sulfur compound having the composition $C_{12}H_{16}N_4SO_2$, which was considerably nearer the truth than the estimate of the Dutch investigators.

The presence of sulfur in the molecule had, however, been reported the previous year by Windaus and his collaborators (12), who obtained the product from yeast by the use of additional methods developed in the intervals by others. Most important of these was the benzoylation of the fuller's eluate according to Seidell (13). This serves to convert a large number of amino compounds into insoluble benzoyl derivatives, leaving the vitamin in a much purer solution. The vitamin hydrochloride as isolated agreed fairly well in physiological and physical properties with Jansen and Donath's description. Fairly consistent analyses were obtained on the salts in the following year (14). A crystalline product of similar description was also obtained in England in 1933 by the charcoal adsorption process (15). The melting points of both the hydrochloride and picrolonate were low, due, as we now believe, to the then unknown dimorphism of these salts.

Further improvements were presently reported in Japan (16) and in the United States (17). Ohdake followed the method of Jansen and Donath essentially, but Williams incorporated several improvements, notably the elution of the vitamin from fuller's earth with quinine acid sulfate instead of baryta, as well as the utilization of benzoylation for purification. Largely due to these expedients, the yields were greatly improved so that sufficient crystalline material became available for chemical study. Later, in England, improved yields by the charcoal method were reported (18).

It is noteworthy that such a series of successes should follow each other so closely but not till several years had elapsed after the original success of the Dutch. Since that time the method has undergone such refinement as to increase the yields still further, as well as to shorten greatly the process. The introduction of synthetic zeolite instead of fuller's earth as an adsorbent offers considerable possibilities, as elution from the zeolite can be accomplished with cold, strong neutral salt solutions (19, 20, 21). The principal interest, however, in the method now lies in the possibility of adapting it to quantitative purposes for the chemical assay of foodstuffs for their thiamin content.

A feature of laboratory technique which was found very useful in the authors' experience may be helpful to others engaged in like work. It was employed throughout the later stages of the isolation procedure and also throughout the steps of cleavage

which led eventually to the establishment of structure. A hint which furnished the basis of the method was obtained from the papers of Kinnersley and Peters. It consists in carrying out all small-scale operations in centrifuge tubes, usually the ordinary 15 c.c. urine centrifuge tube. A stock of suitably marked, tared tubes is kept in readiness. If the evaporation of a solution is to be carried out, the liquid is put in a tube fitted with a two-hole rubber stopper (ground-glass stoppers are preferred for special purposes) according to Fig. 1. Air is admitted through a calcium chloride tube to *A*, and *B* is connected to vacuum. By cautious adjustment of the screw-clamp at *A*, as well as of the opening of the vacuum valve, rapid evaporation may be induced at low temperature and at reduced pressure. It is important that the incoming gentle stream of air be directed on the surface of the liquid so as to cause a slight ripple which greatly hastens evaporation. The tube should be surrounded by a water bath kept

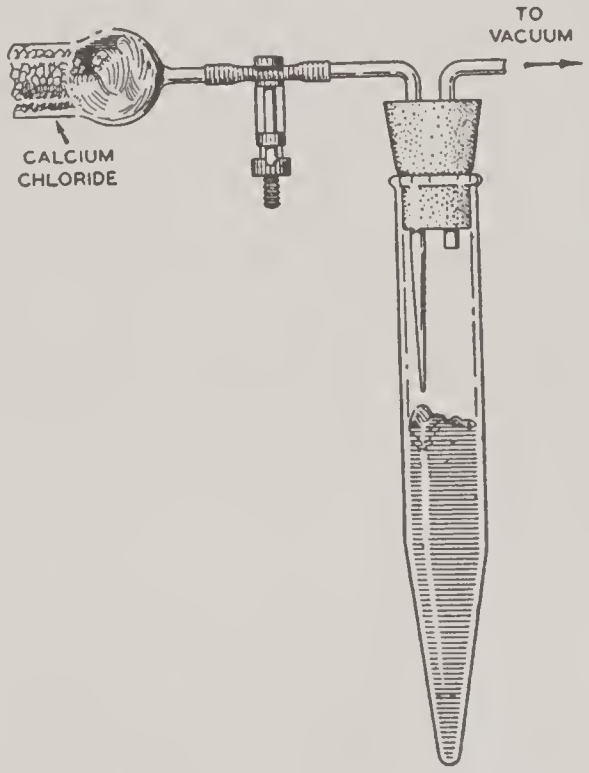


FIGURE 1. Device for Quantitative Manipulation of Small Amounts of Substance.

at the desired temperature. Ten cubic centimeters of water can be evaporated in an hour or, if the liquid is a more volatile one, in correspondingly less time.

If, during the course of evaporation, a precipitate separates, it may be deposited by centrifugation and the clear liquid decanted for further evaporation in another tared tube. The precipitate may be dried in the same way and weighed. If a crop of crystals separates during evaporation, they may be washed down to the bottom of the tube by suitable rotation and tilting of it,

and packed by centrifuging. The mother liquor may then be decanted and the crystals washed and dried in the tube in the same way. It has been our practice systematically to record the dry weights of all fractions obtained in this way. It is often of great value to be able to account for every milligram of the starting material, as the appearance of deposits is often very deceptive as a means of estimating whether or not they are negligible in weight. When dealing with a new substance, various solvents may be tried successively without any loss of material. A solvent which proves unsuitable is quickly evaporated and replaced by another. As compared with a flask and fibulator or an open dish in a vacuum desiccator for conducting vacuum evaporations, the above method not only avoids loss by spattering, creepage of solution, excessive dilution in transferring from one vessel to another, etc., but it is also much more rapid. If a vacuum distillation or sublimation of the substance itself is to be used as the final means of purification, a centrifuge tube, with an interchangeable ground-glass joint, may be used first for the evaporation of solvent and later as a sublimation apparatus by substituting a suitable condenser for the above "rippler" connection.

By this device we were able, starting with 27 mg. of the aminosulfonic acid from the vitamin, to carry out its reduction with sodium in liquid ammonia and to isolate from the product sufficient 2,5-dimethyl-6-aminopyrimidine to secure carbon, hydrogen, and nitrogen determinations on the free base as well as on the picrate, together with melting points of both. The method is also serviceable for semi-microsyntheses with 5 to 50 mg. of each reactant.

BIBLIOGRAPHY

1. Fraser, H., and Stanton, A. T.: Etiology of Beriberi. Studies from Inst. Med. Research, Fed. Malay States, No. 12, 1911.
2. Funk, C.: The Chemical Nature of the Substance Which Cures Polyneuritis in Birds Induced by a Diet of Polished Rice. Jour. Physiol. *43*, 395, 1911.
3. Chamberlain, W. P., and Vedder, E. B.: A Contribution to the Etiology of Beriberi. Philip. Jour. Sci. Sect. B, *6*, 251, 1911.
4. Edie, C. S., Evans, W. H., Moore, B., Simpson, G., and Webster, A.: Antineuritic Bases of Vegetable Origin in Relationship to Beriberi, with a Method of Isolation of Torulin, the Antineuritic Base of Yeast. Biochem. J. *6*, 234, 1912.
5. Suzuki, Shimamura, T., and Ohdake, S. Über Oryzanin. Biochem. Z. *43*, 89, 1912.
6. Seidell, A.: A Stable Form of Vitamine Efficient in the Prevention

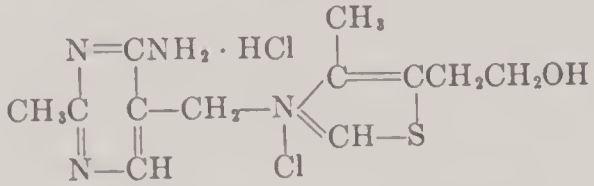
and Cure of Certain Deficiency Diseases. U. S. Public Health Repts. *31*, 364, 1916.

7. Williams, R. R.: The Chemical Nature of the Vitamins. Antineuritic Properties of the Hydroxypyridines. Jour. Biol. Chem. *25*, 437, 1916. The Structure of the Curative Modifications of the Hydroxypyridines. Jour. Biol. Chem. *29*, 495, 1917. Vitamins from the Standpoint of Structural Chemistry. Jour. Ind. Eng. Chem. *13*, 1107, 1921.
8. Hofmeister, E.: Zur Kenntnis der alkaloidischen Bestandteile des Reiskleie. Biochem. Z. *103*, 218, 1920.
9. Peters, R. A.: The Action of Nitrous Acid upon the Antineuritic Substances in Yeast. Biochem. J. *18*, 858, 1924.
10. Jansen, B. C. P., and Donath, W. F.: Antineuritisch Vitamine. Chem. Weekblad. *23*, 201, 1926. On the Isolation of the Anti-beriberi Vitamin. Proc. Koninklijke Akad. Wetensch., Amsterdam, *29*, 1390, 1926. Isolation of Anti-beriberi Vitamin. Mededeel. Dienst. Volksgezondheid. Nederland. Indie. *16*, 186, 1926.
11. Ohdake, S.: Isolation of Oryzanin, "Antineuritic Vitamin," from Rice Polish. Bull. Agric. Chem. Soc. Japan *8*, 179, 1932.
12. Windaus, A., Tschesche, R., Ruhkopf, H., Laquer, F., and Schultz, F.: Die Darstellung von kristallisiertem antineuritischem Vitamin aus Hefe. Nach. v. der Ges. Wiss. Göttingen III, 207, 1931.
13. Seidell, A.: Further Progress toward the Isolation of the Antineuritic Vitamin (Vitamin B) from Brewers' Yeast. Jour. Biol. Chem. *82*, 633, 1929.
14. Windaus, A., Tschesche, R., and Ruhkopf, H.: Über das antineuritische Vitamin. Nach. v. der Ges. Wiss. Göttingen III, 344, 1932.
15. Kinnersley, H. W., O'Brien, J. R., and Peters, R. A.: Crystalline Preparations of B_1 from Bakers' Yeast. Biochem. J. *27*, 232, 1933.
16. Ohdake, S.: Isolation of Oryzanin (Antineuritic Vitamin) from Yeast. Proc. Imp. Acad. Japan *10*, 95, 1934.
17. Williams, R. R., Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin. Jour. Amer. Chem. Soc. *56*, 1187, 1934.
18. Kinnersley, H. W., O'Brien, J. R., and Peters, R. A.: Improved Yields of Vitamin B_1. Biochem. J. *29*, 716, 1935.
19. Cerecedo, L. R., and Hennessy, D. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. I. Rice Polishings. Jour. Amer. Chem. Soc. *59*, 1617, 1937.
20. Cerecedo, L. R., and Kaszuba, F. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. II. Brewers' Yeast. Jour. Amer. Chem. Soc. *59*, 1619, 1937.
21. Cerecedo, L. R., and Thornton, J. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. III. Wheat Germ. Jour. Amer. Chem. Soc. *59*, 1621, 1937.

CHAPTER XII

STRUCTURE AND SYNTHESIS OF THIAMIN

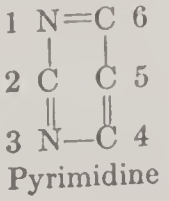
THIAMIN has the following structure (1–9):



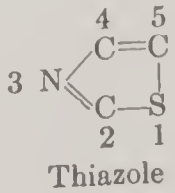
Thiamin Chloride Hydrochloride

It is a unique compound in several particulars, notably in that it contains the thiazole nucleus which is otherwise unknown in nature. However, synthetic thiazole compounds have long been quite familiar. Some of them have attained considerable commercial importance as accelerators for the vulcanization of rubber and as sensitizing dyes in photographic plates. Thiamin is the only one of the known vitamins which contains sulfur, whence this name for it. A number of other sulfur compounds have a conspicuous place in biochemistry, *e.g.*, cystine, cystein, glutathione, ergothioneine, biotin, and insulin, but none of them has an evident relationship to thiamin which is associated in any way with their sulfur atoms.

The pyrimidine nucleus which thiamin contains is a very familiar feature in biochemistry, being present in uric acid and in the considerable group of purines found extensively in plant and animal tissues. Uracil, cystosine, and thymine are simpler pyrimidines which also have a very general occurrence together with purines among the hydrolytic products of the nucleic acids. These latter are compounds of nearly universal occurrence in cells and consist of phosphoric acid, pentose sugars, purines, and pyrimidines in combination. However, all of the other known purines and pyrimidines differ from the pyrimidine of thiamin in that the latter is the sole example of a natural pyrimidine which is alkylated in position 2. The conventional method of numbering the atoms of the pyrimidine and thiazole nuclei is as follows:



Pyrimidine



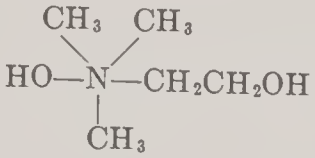
Thiazole

It may be helpful at this juncture to call attention to the fact that position 4 and position 6 in the pyrimidine ring are equivalent, so that 4 amino pyrimidine and 6 amino pyrimidine, for example, are identical substances. If this point is overlooked, the reader who is not intimately familiar with organic chemistry may be confused by the variation in terminology used in the literature cited below.

The pyrimidine of thiamin is also somewhat peculiar in being alkylated also in position 5, a distinction which it shares with thymine. Few pyrimidines alkylated in positions 2 or 5 were known even as synthetic compounds before interest was aroused in them in connection with the study of thiamin. These methyl groups confer somewhat distinctive properties upon this class of pyrimidines. This fact, as well as the rarity of examples of the class, constituted very serious obstacles to the determination of the structure of thiamin, although a pyrimidine nucleus in it had been suspected for years. Thiamin is not the sole vitamin containing the pyrimidine nucleus. It is also present in vitamin B_2 or riboflavin (see page 132), but in non-alkylated form, and it seems quite unlikely that thiamin derives from riboflavin or vice versa.

In another respect, however, there is a resemblance between riboflavin and thiamin which may well be significant, namely, the presence of an alkyl side chain with a terminal hydroxyl group. In riboflavin this side chain is a sugar group attached to the central pyridazine nucleus in the molecule; in thiamin it is an ethyl alcohol on the thiazole nucleus.

Another distinctive feature of thiamin is its character as a quaternary base. This feature is not very prevalent in nature but is present in choline, a substance of considerable biological importance. Choline has the structure



and is also like thiamin in being a derivative of ethyl alcohol. There is a physiological association of choline and thiamin with reference to disposition of fat in the liver (see page 283). The acetyl derivative of choline occurs in the body and appears to possess a definite hormone function. It produces marked dilation of blood vessels and contraction of the muscles. The coferment of Warburg and cozymase are also quaternary nitrogen compounds (see page 162).

Perhaps one should also mention the possibly suggestive but at present quite unintelligible fact that luminal, veronal, and similar hypnotics are pyrimidines which are dialkylated in position 5. One cannot attach much importance to these resem-

blances to known drugs, as thiamin has no pharmacological action in physiological quantities or even in much larger amounts (see page 82).

The main features of the experiments whereby the structure of thiamin was established are noted in the following paragraphs. The story begins with the isolation of the substance as the chloride by Jansen and Donath in 1926. As recounted in the previous chapter, they obtained it from rice polish in what appears to have been substantially pure form, but in amounts inadequate for thorough chemical study, and had the misfortune to overlook completely the presence of sulfur in it. This fact was discovered by Windaus and collaborators in 1932 (10), who also first proposed in effect the proper empirical formula, $C_{12}H_{18}N_4OSCl_2$.

There remained, however, sufficient discordances in the analyses, in reported physiological activities (11), and in absorption spectra (12) that the elementary composition remained somewhat uncertain until 1934, when the availability of larger supplies of the pure vitamin made possible additional analyses (13). The analytical uncertainties centered principally about the fact that the substance is difficult to analyze properly for nitrogen (14) and that the various salts of the vitamin were obtained apparently sometimes with and sometimes without water of crystallization.

The first significant cleavage products of the vitamin were two obtained by the action of nitric acid on the vitamin as reported by Windaus and coworkers (15). Of the two, one was, in all probability, correctly surmised to be an etherified derivative of an alkylated dioxypyrimidine, the etherification being due to the use of ethyl alcohol as a solvent in the isolation. Such an etherification of an oxypyrimidine under extremely mild conditions seemed surprising but has been rendered plausible by later experience. This oxidation product, however, has not yet been reproduced synthetically. The second cleavage product has since been reproduced, but its nature was not recognized at the time, although its composition was determined as $C_5H_5NSO_2$. Windaus and associates also reported at a later time the picrate of another then unknown base of the composition $C_6H_{10}N_4$ (16), which was obtained from the vitamin by oxidation with barium permanganate.

Our knowledge of the structure of thiamin follows largely from the fact that it is quantitatively split by neutral sulfite solution at room temperature according to the equation (17, 18):

$$(1)\quad C_{12}H_{18}N_4SOCl_2 + Na_2SO_3 \longrightarrow \underset{\text{I}}{C_6H_9N_3SO_3} + \underset{\text{II}}{C_6H_9NSO} + 2\,NaCl$$

Product I yields ammonia and a second derivative III, on strenuous hydrolysis with acids (19):

(2) $$C_6H_9N_3SO_3 + H_2O \longrightarrow C_6H_8N_2SO_4 + NH_3$$
III

On treatment with water at 200° C., I (or III) yields sulfuric acid; with alkali, sulfurous acid, thus proving the presence of a sulfonic group in both. Both I and III exhibit absorption in the ultraviolet suggestive of a pyrimidine nucleus. This suggestion was later confirmed by converting I into 2,5-dimethyl-6-aminopyrimidine, IV, by the action of metallic sodium in liquid ammonia (1, 5):

(3) $$C_6H_9N_3SO_3 + 2\,Na \xrightarrow[NH_3]{\text{liq.}} CH_3 \cdot \underset{\underset{N-CH}{\|}}{\overset{N=C\cdot NH_2}{C}} = \underset{\underset{}{}}{\overset{}{C}} \cdot CH_3 + Na_2SO_3$$
IV

IV was previously unknown, but its synthesis was affected by a method adapted from the literature such as to establish its structure (5). Comparison with the substance obtained from the vitamin served to establish their identity.

By a similar synthesis there was obtained (6) 2-methyl-5-ethoxymethyl-6-oxypyrimidine, V, which on treatment with sodium sulfite yielded 2-methyl-6-oxypyrimidine-5-methyl sulfonic acid, VI (equation 4), which proved identical with III (equation 2), whence one could reconstruct the primary cleavage product, I (equation I), as having structure VII.

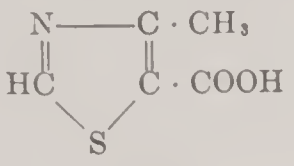
(4) $$CH_3 \cdot \underset{\underset{N-CH}{\|}}{\overset{N=C\cdot OH}{C}} = \underset{}{\overset{}{C}} \cdot CH_2 \cdot OC_2H_5 \xrightarrow{Na_2SO_3} CH_3 \cdot \underset{\underset{N-CH}{\|}}{\overset{N=C\cdot OH}{C}} = \underset{}{\overset{}{C}} \cdot CH_2 \cdot SO_3H$$
V VI

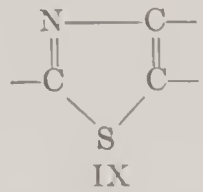
$$CH_3 \cdot \underset{\underset{N-CH}{\|}}{\overset{N=C\cdot NH_2}{C}} = \underset{}{\overset{}{C}} \cdot CH_2 \cdot SO_3H$$
VII

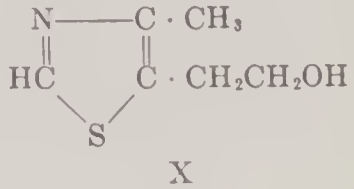
The structure of one of the two main fragments of the vitamin was thus established.

The other fragment, II (equation I), was more readily identified. On oxidation with nitric acid (20) it yielded 4-methylthiazole-5-carboxylic acid, VIII, a substance which was first prepared synthetically in 1890 (21). The same substance had also been obtained by Windaus, Tschesche, and Grewe (15) by oxidizing thiamin itself with nitric acid. Windaus, however, failed to recognize the thiazole nucleus, IX, which it contained. Recognition of this nucleus (22) by the peculiar stability of its sulfur atom was due to Dr. H. T. Clarke, who kindly collaborated at this stage of the study. The thiazole nucleus had never before been found in nature, and its occurrence in thiamin is to be re-

garded as highly significant in a biochemical sense. With knowledge of the presence of this nucleus and with the further information that II (equation I) contained in a side chain an alcoholic hydroxyl group which could be readily replaced by nonionic chlorine without alteration of the nucleus (23), it was a relatively simple matter to reconstruct the remaining vitamin fragment, II (equation I), as having the structure X. It is called 4-methyl-5-(β-hydroxy)ethylthiazole.


VIII

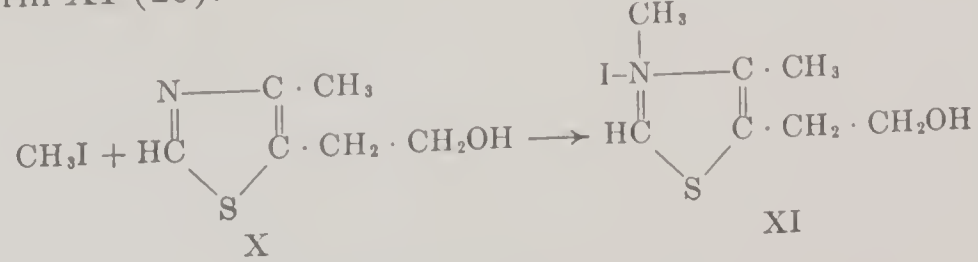

IX


X

In the identification of the cleavage products of thiamin, use was made to an unusual extent of comparisons of ultraviolet absorptions. This was possible because such a large proportion of all the atoms of the thiamin molecule are comprised in the two unsaturated nuclei; viz., pyrimidine and thiazole. Such nuclei have somewhat distinctive absorptions in the ultraviolet. Another physical method, that of potentiometric titration with alkali, was destined to disclose the mode of linkage of the two nuclei to each other.

The point of attachment on the pyrimidine portion was marked by the position of the sulfonic group which was introduced during the sulfite cleavage of thiamin. The point of attachment on the thiazole was less conspicuously marked. It was identified in the following way: Thiamin chloride on being titrated with alkali behaved in a peculiar manner. After the addition of 1 mole of alkali, the alkalinity of the solution no longer rose steadily with further additions (24). Rather, the alkalinity arose only temporarily after each addition and then fell again during the course of a few minutes, indicating an internal rearrangement of the molecule taking place not instantly, as ionic reactions do, but relatively slowly. Furthermore, this deportment continued until a total of 3 moles of alkali had been added. Only then was there a marked and steady rise in alkalinity. The excessive proportion of alkali required was wholly unexpected at the time, as thiamin was known to contain only two acid radicals.

It had been found possible to add methyl iodide to compound X to form XI (20).

(5) 
X XI

Such an addition of methyl iodide is a very general property of tertiary nitrogenous bases and was therefore to be expected in this case. On titration of XI with alkali it showed the same peculiar behavior as thiamin (see Fig. 2), thus pointing to a fundamental kinship which could be accounted for only by the structure assigned to thiamin shown on page 144.

As a matter of interest as to technique, ultraviolet absorption curves were found very useful in working out the structure of thiamin, as so large a proportion of its atoms are contained in the two nuclei. These, like other unsaturated ring structures in

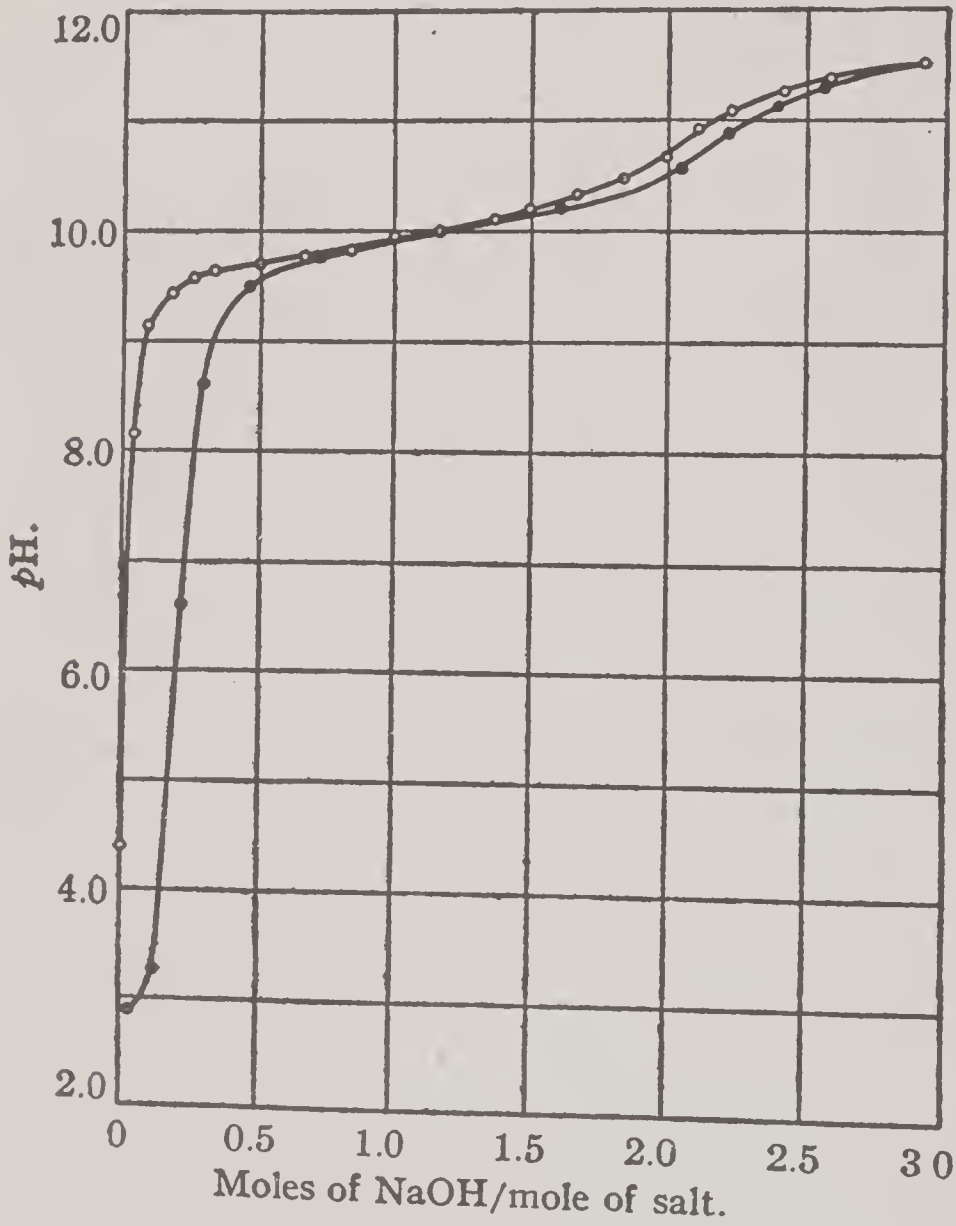


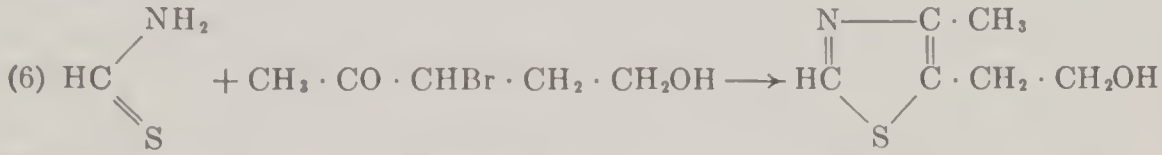
FIGURE 2. Titration of methiodide of basic cleavage product: o—o, titration with NaOH; •—•, back titration with HCl. Compare with Figure 6, p. 163. Courtesy of *Journal of American Chemical Society*.

general, give characteristic absorption in the region 230 to 300 mu. Such studies were especially useful in dealing with the pyrimidine as the amino cleavage products, like all the 6 amino

pyrimidines, give absorption which is single-banded in acid solution, but which becomes double-banded in alkali. (See Figs. 3 and 4.)

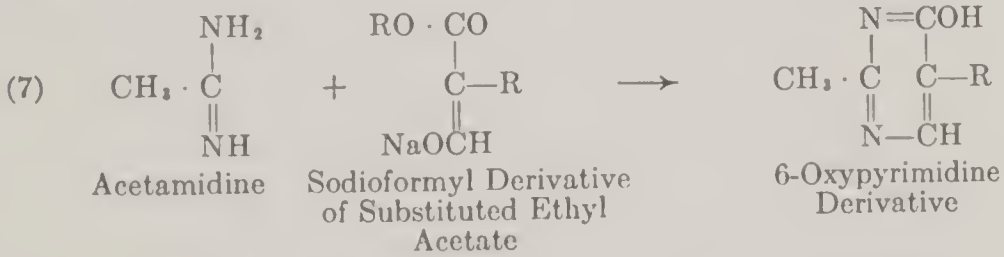
Synthesis of Thiamin

The first step in the synthesis of thiamin was the reproduction of the thiazole portion. This was done by Buchman by the simple reaction:

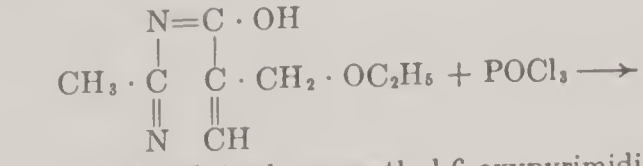


Other syntheses of the same compound also have been achieved (22).

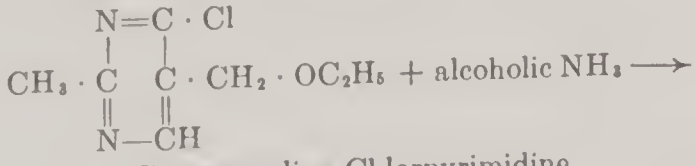
The synthesis of the pyrimidine portion was accomplished by methods similar to those employed for the production of pyrimidines IV, V, and VI, previously discussed in connection with the establishment of structure. The general method is as follows (2, 6):



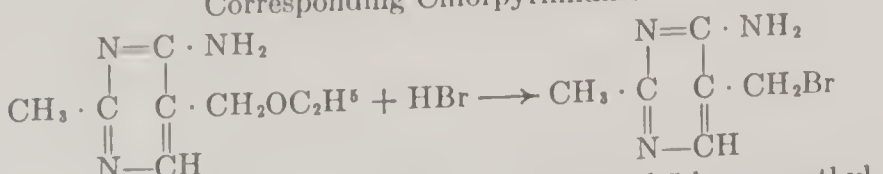
If in the foregoing sodioformyl derivative R represents CH_3, the pyrimidine obtained is 2,5-dimethyl-6-oxypyrimidine; if R represents $CH_2OC_2H_5$, the resulting pyrimidine is V (equation 4). For the synthesis of thiamin itself, product V is converted into a brom-amino derivative, thus:



V. (2-Methyl-5-ethoxy-methyl-6-oxypyrimidine)



Corresponding Chlorpyrimidine



Corresponding Aminopyrimidine

(2-Methyl-5-bromomethyl-6-aminopyrimidine)

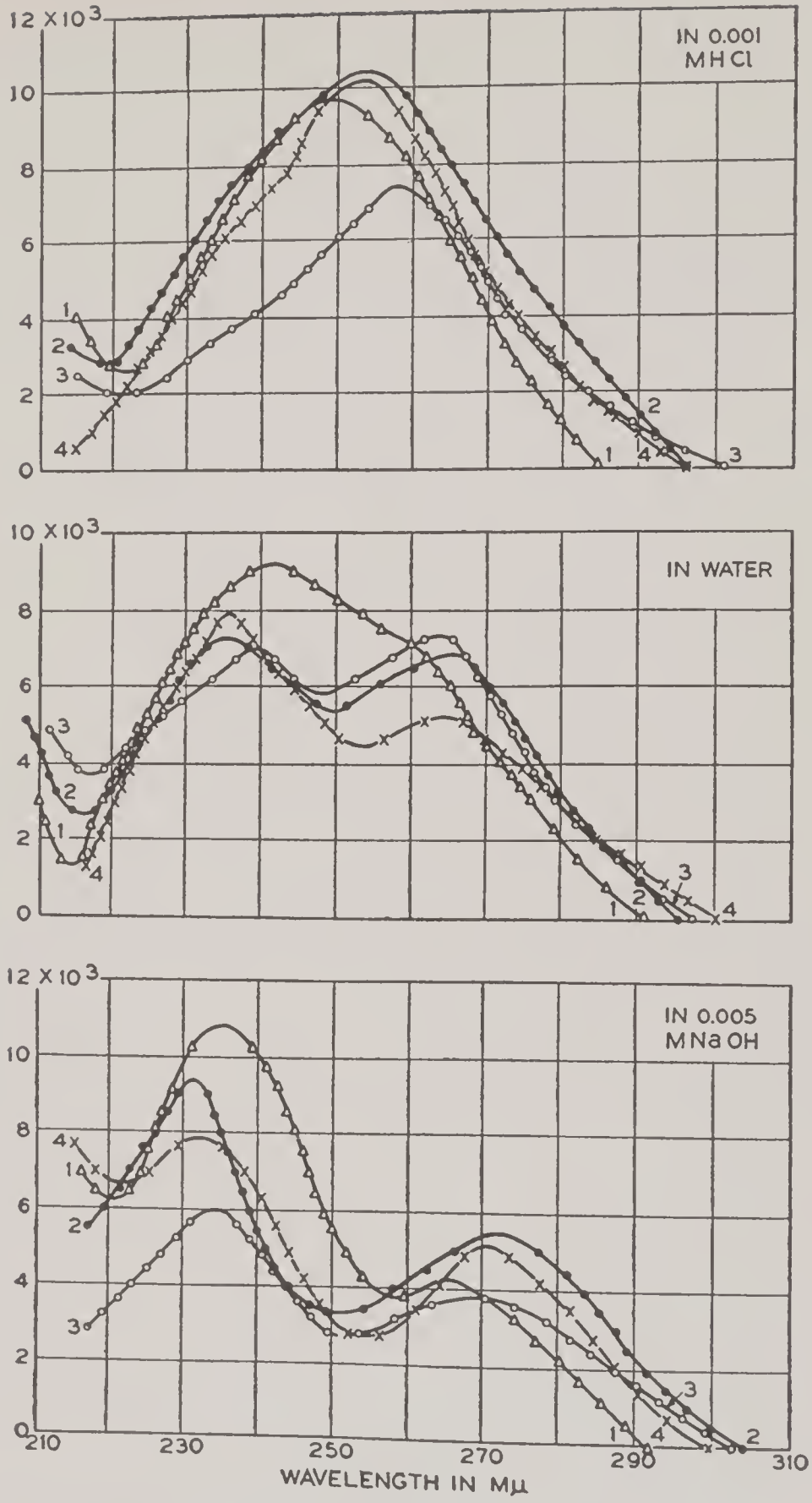


FIGURE 3. Ultraviolet absorption of 6-amino-pyrimidines.

1. 2,4-dimethyl-6-amino-pyrimidine
2. 2,5-dimethyl-6-amino-pyrimidine
3. 4,5-dimethyl-6-amino-pyrimidine
4. 5-ethyl-6-amino-pyrimidine

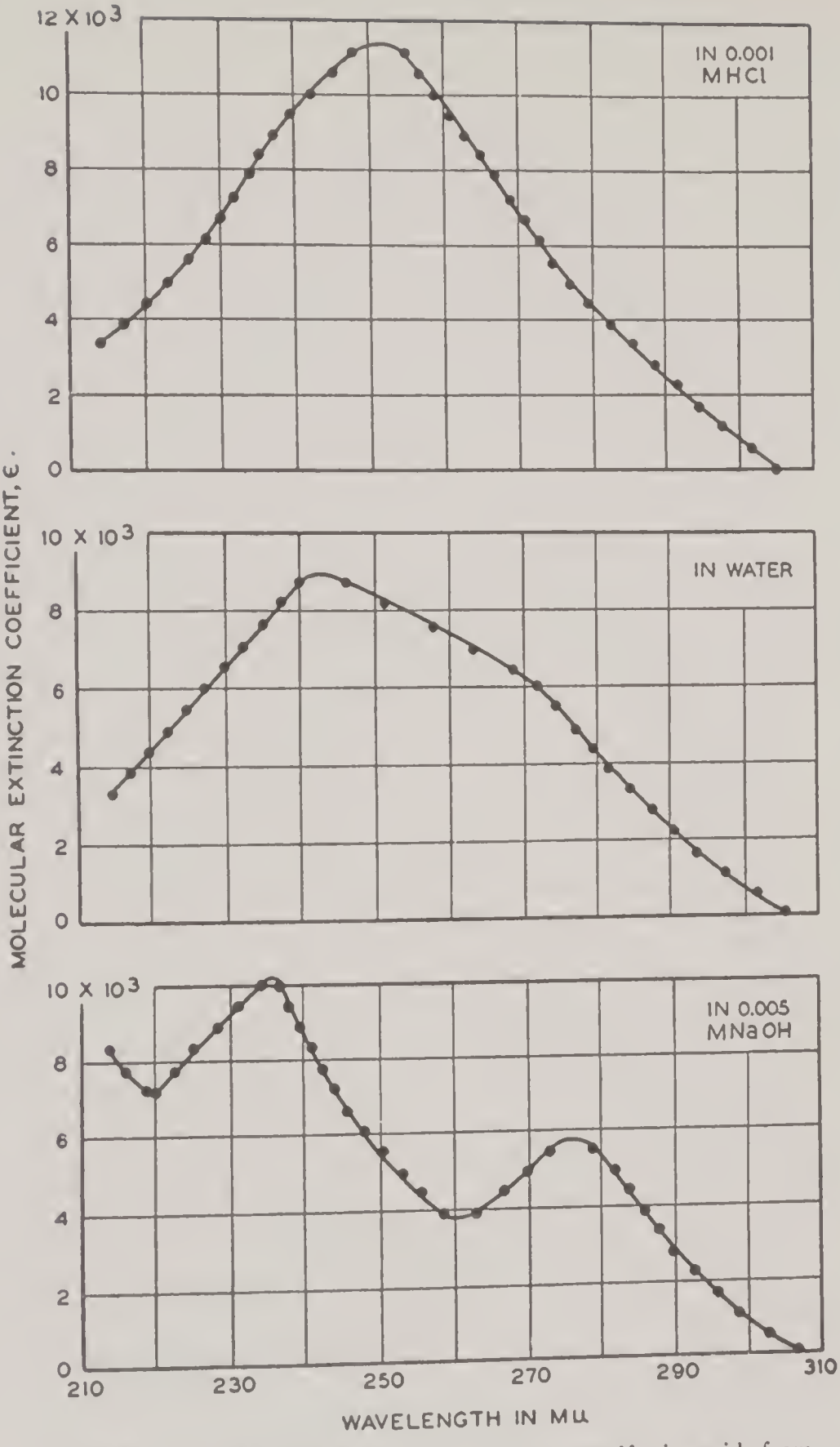


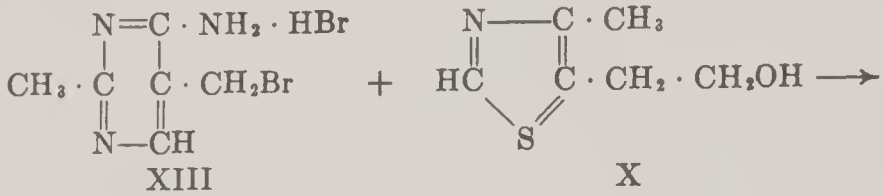
FIGURE 4. Ultraviolet absorption of amino-sulfonic acid from vitamin.

Figures 3 and 4 by courtesy of *Journal of American Chemical Society*.

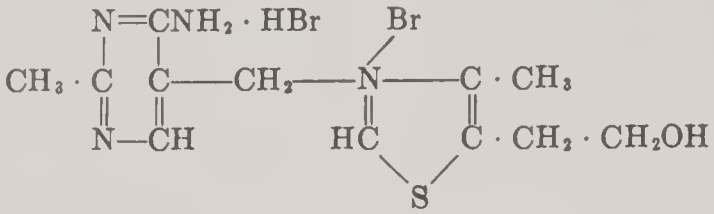
The last of this series of steps is somewhat analogous to that of equation 4. The purpose of putting the bromine in the side chain in position 5 instead of the sulfonic group is that the bromo compound, XIII, is very reactive, while the corresponding sulfonic compound, VII, is extremely unreactive.

The final step in the synthesis of thiamin is to heat the hydrobromide of the bromopyrimidine, XIII, with the thiazole, X. This results in combination of the two molecules to form thiamin bromide hydrobromide, a reaction quite analogous to that shown in equation 5.

(8)



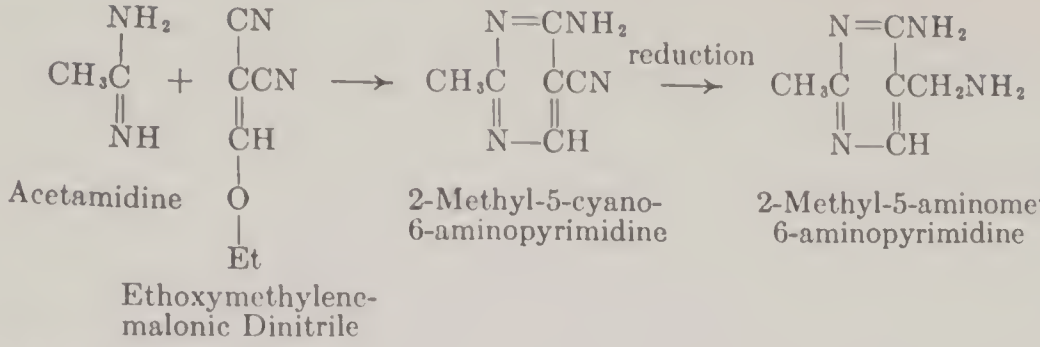
XIII X



XIV. Thiamin Bromide Hydrobromide

The bromide hydrobromide, XIV, is readily converted into the chloride hydrochloride (page 166) simply by shaking a solution of the former with silver chloride. The resulting substance is chemically identical with the thiamin chloride previously obtained from nature (25). The correspondence of the ultraviolet absorption spectra of the two synthetic salts with that from nature is shown in Figure 5. Its physiologic action is also identical (26). As first obtained, the vitamin chloride hydrochloride melted at 232° to 234° rather than at 246°–250°; as had been commonly observed of the product obtained from nature. However, it was later found possible to convert the low-melting form into the high-melting one (25) and ample evidence has accumulated that this crystalline dimorphism is common to several salts of both the natural and synthetic product. The identity of the synthetic and natural substance has been fully established by many workers and has not been in dispute.

A day or two before the above-described synthesis was published schematically in America a paper by Grewe (3) was published in Germany announcing the same structure which had appeared only seven weeks previously in this country. Grewe synthesized in the following manner (27) the diamino cleavage product previously obtained from the vitamin by Windaus and associates as the picrate of an unknown base (16).



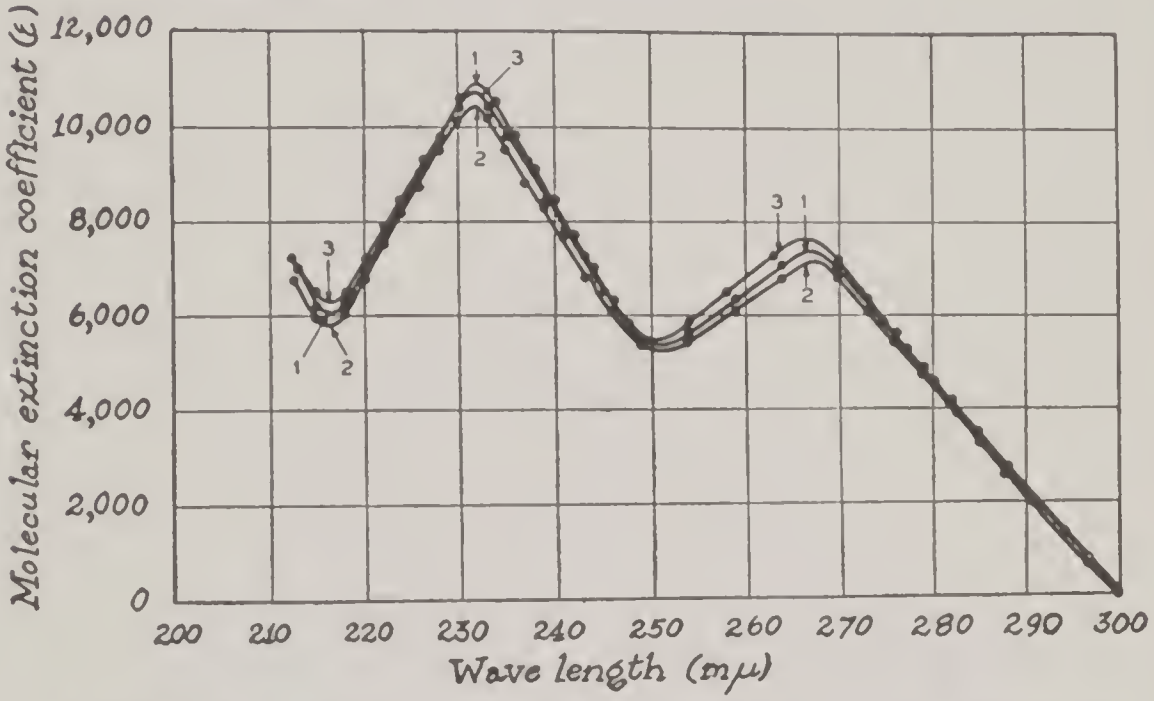
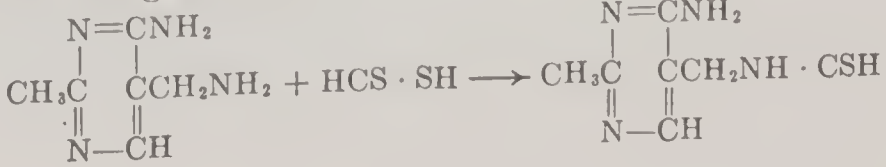


FIGURE 5. A comparison of the ultraviolet absorptions of the natural and synthetic products: curve 1, natural vitamin (hydrochloride); curve 2, synthetic vitamin (hydrochloride); curve 3, synthetic vitamin (hydrobromide). Courtesy of *Journal of the American Medical Association.*

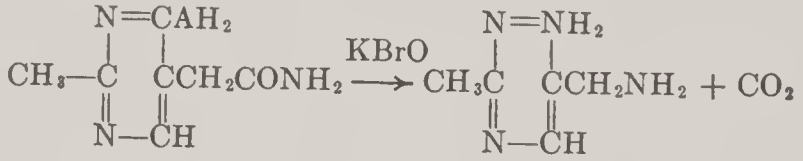
From the diamino compound he obtained by the action successively of nitrous acid and of hydrobromic acid the 5-bromomethyl derivative and from treatment of it with sodium sulfite the amino sulfonic acid, I, previously derived by Williams *et al.* by sulfite cleavage of the vitamin. Grewe also stated that he had learned that a synthesis of the vitamin had been effected recently using intermediates made by methods similar to his own by Andersag and Westphal at the Elberfeld Laboratories of the I. G. Farbenindustrie.

In the meantime, however, Todd and Bergel, who had contributed several important papers relating to thiochrome, and thus had aided considerably in establishing the structure of thiamin, carried out in England a new synthesis of thiamin (4) by the following route:



The thioformyl group of this pyrimidine was then converted to a thiazole nucleus by condensation with chloracetopropyl ether in a manner analogous to the condensation of bromacetopropyl alcohol with thioformamide (see page 150). The resulting ether of thiamin was readily converted to thiamin by hydrolysis. The thiochrome studies of these authors and their associates will be discussed in Chapter XIV.

The details of the American synthesis were published early in 1937 (6). That of Andersag and Westphal which was referred to by Grewe has since appeared (9). These authors disclose two schemes following essentially the same lines as the two previously published syntheses of Williams and Cline and of Todd and Bergel. They claim independent discovery of both methods and disclose the synthesis of thiamin by these methods as well as a number of analogues and derivatives of it which will be discussed in Chapter XIV. As to the synthesis of the intermediates necessary for the production of thiamin, the German workers revealed the Hofmann degradation of the appropriate pyrimidyl acetamide, a procedure which both the American and British workers had tried unsuccessfully as reported in their last above cited papers. This appears to be the best method of synthesis of the diamino pyrimidine which was first prepared synthetically by Grewe. The work has not yet been confirmed by others. The reaction follows:



It frequently happens that the precise structure of a complicated organic compound remains in dispute for a long time because the findings of cleavage reactions are not clear or even because the methods of synthesis are ambiguous. Fortunately in the instance of thiamin, confirmation has come almost immediately from numerous sources.

BIBLIOGRAPHY

1. Williams, R. R.: Structure of Vitamin B_1. Jour. Amer. Chem. Soc. *58*, 1063, 1936.
2. Williams, R. R., and Cline, J. K.: Synthesis of Vitamin B_1. Jour. Amer. Chem. Soc. *58*, 1504, 1936.
3. Grewe, R.: Über das antineuritische Vitamin. Zeit. f. physiol. Chem. *242*, 89, 1936.

4. Todd, A. R., and Bergel, F.: Aneurin Part VII. A Synthesis of Aneurin. Jour. Chem. Soc. Lond. p. 364, 1937.
5. Cline, J. K., Williams, R. R., Ruehle, A. E., and Waterman, R. E.: Studies of Crystalline Vitamin B_1. XVI. Identification of the Pyrimidine Portion. Jour. Amer. Chem. Soc. *59*, 530, 1937.
6. Cline, J. K., Williams, R. R., and Finkelstein, J.: Studies of Crystalline Vitamin B_1. XVII. Synthesis of Vitamin B_1. Jour. Amer. Chem. Soc. *59*, 1052, 1937.
7. Ohdake, S., and Yamagishi, T.: Oryzanin, "Antineuritic Vitamin B." On the Chemical Constitution of "Oryzanin." Bull. Agric. Chem. Soc. Japan *13*, 47, 1937.
8. Imai, T.: The Chemistry of Vitamin B_1. J. Biochem. (Japan) *25*, 95, 1937.
9. Andersag, H., and Westphal, K.: Über die Synthese des antineuritischen Vitamins. Ber. deut. chem. Ges. *70*, 2035, 1937.
10. Windaus, A., Tschesche, R., and Ruhkopf, H.: Über das antineuritische Vitamin. Nach. v. der Ges. der Wiss. Göttingen III, 342, 1932.
11. Kinnersley, H. W., O'Brien, J. R., and Peters, R. A.: Crystalline Preparations of Vitamin B_1 from Bakers' Yeast. Biochem. Jour. *27*, 232, 1933.
12. Windaus, A., Tschesche, R., Ruhkopf, H., Laquer, F., and Schultz, F.: Die Darstellung von kristallisiertem antineuritischem Vitamin aus Hefe. Zeit. physiol. Chem. *204*, 123, 1932.
13. Wintersteiner, O., Williams, R. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B_1. II. Elementary Composition and Ultraviolet Absorption. Jour. Amer. Chem. Soc. *57*, 517, 1935.
14. Hayman, D. F., and Adler, S.: Microanalysis of Nitrogen in Certain Pyrimidines by the Dumas Method. Ind. Eng. Chem. *9*, 197, 1937.
15. Windaus, A., Tschesche, R., and Grewe, R.: Über das antineuritische Vitamin. Zeit. physiol. Chem. *228*, 27, 1934.
16. Windaus, A., Tschesche, R., and Grewe, R.: Über das antineuritische Vitamin. Zeit. physiol. Chem. *237*, 98, 1935.
17. Williams, R. R.: Structure of Vitamin B_1. Jour. Amer. Chem. Soc. *57*, 229, 1935.
18. Williams, R. R., Waterman, R. E., Keresztesy, J. C., and Buchman, E. R.: Studies of Crystalline Vitamin B_1. III. Cleavage of Vitamin with Sulfite. Jour. Amer. Chem. Soc. *57*, 536, 1935.
19. Williams, R. R., Buchman, E. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B_1. VIII. Sulfite Cleavage. Chemistry of the Acidic Product. Jour. Amer. Chem. Soc. *57*, 1093, 1935.
20. Buchman, E. R., Williams, R. R. and Keresztesy, J. C.: Studies of Crystalline Vitamin B_1. X. Sulfite Cleavage, Chemistry of the Basic Product. Jour. Amer. Chem. Soc. *57*, 1849, 1935.
21. Wohmann, M.: Die "Diazoverbindungen" der Thiazolreihe und ihre Reactionen. Ann. d. Chem. *259*, 299, 1890.

22. Clarke, H. T., and Gurin, S.: Studies of Crystalline Vitamin B_1. XII. The Sulfur-containing Moiety. Jour. Amer. Chem. Soc. *57*, 1876, 1935.
23. Buchman, E. R., and Williams, R. R.: Studies of Crystalline Vitamin B_1. IX. Action of Concentrated Hydrochloric Acid. Jour. Amer. Chem. Soc. *57*, 1751, 1935.
24. Williams, R. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B_1. XI. Presence of Quaternary Nitrogen. Amer. Chem. Soc. *57*, 1856, 1935.
25. Williams, R. R., and Cline, J. K.: Synthetic Vitamin B_1. Jour. Amer. Chem. Soc. *59*, 216, 1937.
26. Eckler, C. R., and Chen, K. K.: Action of Synthetic Vitamin B_1. Proc. Soc. Exper. Biol. Med. *35*, 458, 1937.
27. Grewe, R.: Die Konstitution des Aneurins (Vitamin B_1). Naturwiss. *24*, 657, 1936.

CHAPTER XIII

REACTIONS, TESTS, AND STANDARDS OF THIAMIN

The knowledge of the structure of thiamin has measurably improved our appreciation of the reasons which underlie its instability in our foods. This is a matter of no small importance in connection with the transport, storage, and processing of food, and applies to home-cooking methods as well as to factory operations. It seems highly probable that the primary cause of instability is the relatively ready splitting apart of the two constituent nuclei, although in the case of alkaline destruction an opening of the thiazole ring is probably involved.

Thermolability and Sensitivity to Alkali

Foremost in importance among the practical causes of vitamin destruction is thermolability. Yet the evidence on this matter is quite conflicting. Numerous instances can be cited from the literature which appear to offer good grounds for believing that loss due to heat applied in the cooking of certain foods, some of them staples such as bread, is almost negligible. On the other hand, instances of very substantial loss from this cause can be found in the case of other articles of diet. A number of epidemics of beriberi in the Orient occurring in institutions have been traced to the introduction of pressure cookers. The reasons for the conflicting evidence are not wholly clear but sufficient possible reasons are evident to account for it. Among these may be mentioned the now rapidly rising evidence that thiamin occurs in tissues partly in the free form and partly as the pyrophosphate (page 345). There may exist marked differences of thermolability between them. However, studies of the thermolability of the pyrophosphate have not yet been reported.

Other relatively recently developed potential causes have to do with possible reactions of the vitamin with other components of the diet. The profound cleaving action of sulfites (page 146) is an example. The amino group of the pyrimidine is said to be capable of reacting readily with aldehydes, presumably to form Schiff's base type of derivatives, and inasmuch as the amino group is essential to physiological activity, this possibility cannot be ignored. Aldehydes are of very common occurrence in foods.

Further numerous coupling reactions of the vitamin have been noted. These are conspicuous because the products are highly colored, but kindred couplings of an inconspicuous character may well occur to destroy activity. The vitamin molecule has several points of possible attack, and inasmuch as all such reactions are promoted by heat, all may contribute to apparent thermolability to varying degree, depending on the components of the foods.

There are, however, some causes of destruction which have long been recognized, namely, heat and alkali. Oxidation, on the other hand, has been ruled out as an important cause. It is comforting to find that study of the pure vitamin tends to confirm these ideas derived from earlier experience. We may well summarize at this point several of the more important studies of thermolability in foods.

Most observers have detected little or no destruction of the vitamin in foodstuffs after exposure to heat at 100° C. Eijkman (1) found no destruction of the properties of unmilled rice after heating at 100° for three hours. Chick and Hume (2) concluded that only slight destruction of the antineuritic vitamin could be detected after heating wheat embryo and yeast extract at 100° C. for one to two hours. With higher temperatures there was a progressively increasing degree and rate of destruction, the original potency being reduced to a quarter or less after two hours at about 129° C. Marked destruction of the antineuritic properties of various foodstuffs, such as meat, eggs, yeast extract, and wheat flour, when heated for varying periods at temperatures of 110°–124° C., has been noted by Holst (3), Vedder (4), and Chick and Hume, but according to Holst, dried peas and whole barley appeared to suffer no loss at 118° C. for ten minutes.

Sherman and Grose (5) investigated the effect of four hours' heating upon vitamin B complex of tomato juice (at its natural acidity, about *p*H 4.3) at 10° intervals over the range of 100°–130°, using the growth of young rats during an experimental period of eight weeks as a measure of vitamin B content. They calculated the rate of destruction to be 20 per cent, 33 per cent, 45 per cent, and 58 per cent at 100°, 110°, 120°, and 130° respectively, a 1.4-fold increase per 10° in comparison with the 2-fold increase in most chemical reactions.

The investigation was extended to tomato juice at *p*H values of 4.28, 5.2, 7.9, 9.2, and 10.9 by Sherman and Burton (6), who found that heating at 100° for four hours produced 20 per cent, 30 per cent, and 70 per cent at *p*H 4.28, 5.2, and 7.9 respectively, and that with heating for one hour at 100° the shift from *p*H 4.28 to 7.9, 9.2, and 10.9 produced increased destruction of 20 to 30 per cent, 60 to 70 per cent, and 90 to 100 per cent respectively.

Experiments with protein-free milk varying the *p*H from 4.3 (natural) to 10 showed the following losses (7):

pH	*Temperature*	*Time*	*Per Cent Destruction*
4.3	97	1 hr.	25
4.3	97	4 hr.	30–40
7	97	1 hr.	70–80
7	97	4 hr.	ca. 100
4.3–7	room	1 wk.	none
10	room	1 wk.	ca. 100

Recently Keenan, Kline, Elvehjem, and Hart (8) have shown that vitamin B_1 in yeast, liver, and in a natural grain ration was destroyed completely by autoclaving and that it was inactivated to a great extent in yeast and liver by heating the foodstuff in the fresh state at 100° C. for 24 hours. It was shown by Guha and Drummond (9) that boiling a concentrated vitamin B_1 preparation for twenty-four hours at *p*H 1 did not cause any appreciable inactivation, while at *p*H 5 about half the activity was destroyed. At *p*H 9 merely boiling for one hour destroyed half of it.

Closely associated with true loss by heat is loss by solution in cooking waters, a point which has been emphasized by Baker and Wright (10). This is, of course, entirely consistent with the well-known extreme solubility of the vitamin in water. Adsorption upon starch in foods probably is an important factor in causing retention of the vitamin. Thus potatoes boiled in the skins retained about nine tenths of the vitamin, only one tenth appearing in the water drained from them. A similar adsorption of vitamin by penetration into the endosperm has been noted in the parboiling of rice. However, many foods, especially those of the more succulent varieties, doubtless have relatively low capacities for adsorption. Cooking waters and juices ought, therefore, to be incorporated systematically in soups, sauces, or gravies for consumption with the main mass of the food. In cooking bread or cereals no water is drained away and losses are trivial.

Baking powder biscuit, however, loses much of its vitamin in cooking, due to induced alkalinity. The widespread practice of using soda to preserve the color of green vegetables during cooking is reprehensible from this standpoint.

All these facts are reasonably consistent with the behavior of the pure vitamin. There is general agreement that the resistance of vitamin B_1 to heat in faintly acid or acid media is considerable. Laboratory tests on vitamin B_1 crystals have shown that, in the range *p*H 3.5–5.0, aqueous solutions are stable for a considerable time. In sealed tubes at 100° and 125°, 0.1 per cent solutions at *p*H 3 and *p*H 6 were unaffected on heating for thirty minutes. Solutions of crystalline vitamin B_1 hydrochloride (*p*H about 3.5) for parenteral injection may be sterilized by heating for one hour at 100° C., or by heating for twenty minutes at

120° C., without appreciable loss in potency; solutions so sterilized remain stable for more than twenty months. The activity of thiamin chloride crystals is totally destroyed by autoclaving at 15 pounds' pressure in alkaline solution for thirty minutes. In the dry form the vitamin is relatively stable. Heating in air at 100° for twenty-four hours induces no loss in potency. Ohdake and Yamagishi (10) reported somewhat higher losses in solution at the natural *p*H, but the amounts of substance then available did not permit very rigorous animal tests. Dry heat for one hour at 170° destroyed 80 per cent of the activity; at 180° 100 per cent.

According to Barger, Bergel, and Todd (12), vitamin B_1, prepared in amorphous form by treating the crystalline hydrochloride with the calculated amount of silver oxide, undergoes decomposition when heated to 180° in a high vacuum, yielding a crystalline solid, $C_9H_{10}N_4O$, which shows in methyl alcoholic solution an intense blue fluorescence, a property which is not possessed by the original vitamin.

Sensitivity to Sulfite

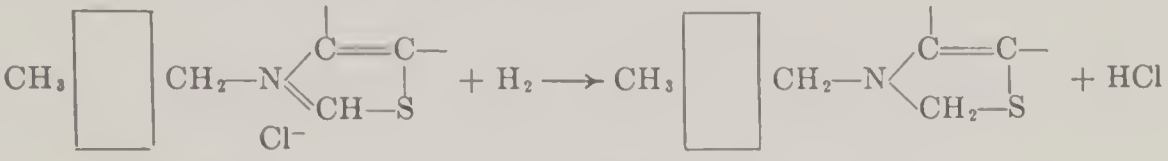
In connection with the sensitivity of the vitamin to alkalinity attention should be called to the effect of *p*H on the sulfite cleavage. This reaction proceeds momentarily at *p*H 6; at *p*H 5 it is complete over night; at *p*H 3 vitamin may be kept in solution with sulfite for months without serious loss of potency (13). This reaction may have some importance as a practical source of loss of vitamin in foods. Morgan (14) has reported loss in the thiamin content of fruits in sulfuring them. An appreciable loss was experienced in rice stored in a ship which had been fumigated with sulfur dioxide (15). Probably the acidity of sulfur dioxide solutions tends to diminish losses. Sulfur dioxide and sulfites are quite toxic to plants and animals, and it seems doubtful whether significant amounts of sulfite can exist in such tissues which are used as food. In chemical processing of extracts, however, sulfur dioxide or sulfites may well be generated at times and constitute a source of loss of activity.

Oxidation-Reduction

Thiamin is not sensitive to atmospheric oxidation. In solution it may be oxidized to thiochrome (page 174). This oxidation has not been shown to be reversible, and as thiochrome is without antineuritic action, it does not appear likely that thiochrome represents a step in the physiological utilization of thiamin but is a degradation product of it.

Lipmann (16) has studied the reduction of thiamin and has thus revealed facts which may have a bearing on the physiological

behavior of the substance. At *p*H 7.5, reduction with platinum-black results in addition of approximately 1 mole of H_2. Reduction by hydrosulfite in sodium carbonate solution, however, is said to follow a different course. One hydrogen atom is added and 1 mole of acid is formed for each mole of thiamin reduced. The reaction is interpreted thus:



That is, 1 H is added to the 2 carbon atom; the other hydrogen loses an electron to neutralize the charge on the complex cation and becomes an H^+ ion. The basis of this interpretation is the apparently similar behavior of the coferments of Warburg and Christian (17) and of von Euler (18). Both of these coferments contain nicotinamide, the nitrogen of the pyridine being in quaternary form. They undergo a reversible reduction of the type indicated above for thiamin. The reduction of the latter, however, has not yet been shown to be reversible.

More recently, distinct greenish-yellow color is reported as a transient phenomenon in the course of the hydrosulfite reduction (19) or in reduction with zinc and hydrochloric acid. As a result, it was suggested that the colored product may be a half-reduced thiazole analogous to the semi-quinones of Michaelis (20). Similar transitory colored products were noted with nicotinic amide methiodide upon reduction. One recalls with curiosity the somewhat less transitory blue color obtained on splitting the vitamin with liquid ammonia (21).

The subject of reversible oxidation reduction of thiamin should be pursued diligently and should be extended to its pyrophosphate as well as the free vitamin. The evidence that thiamin pyrophosphate functions as a decarboxylating enzyme does not invalidate the other evidence that an oxidative catalysis is also often involved (see Chapter XXVI). In association with the proper protein component of an enzyme system, it is still eminently probable that thiamin, or a derivative of it, undergoes reversible oxidation and reduction as a part of its fundamental physiological utilization.

Action of Alkali

The structure of thiamin as finally determined makes possible an understanding of the significance of the peculiar potentiometric titration curve (Fig. 6) of the substance. This is due to a characteristic which appears common to all quaternary thiazoles having H in position 2.

On titrating thiamin chloride hydrochloride with alkali, we find a sharp rise when 1 mole is reached corresponding to the formation of the neutral or mono acid salt, but there is no further break until a total of 3 moles has been added. Apparently the

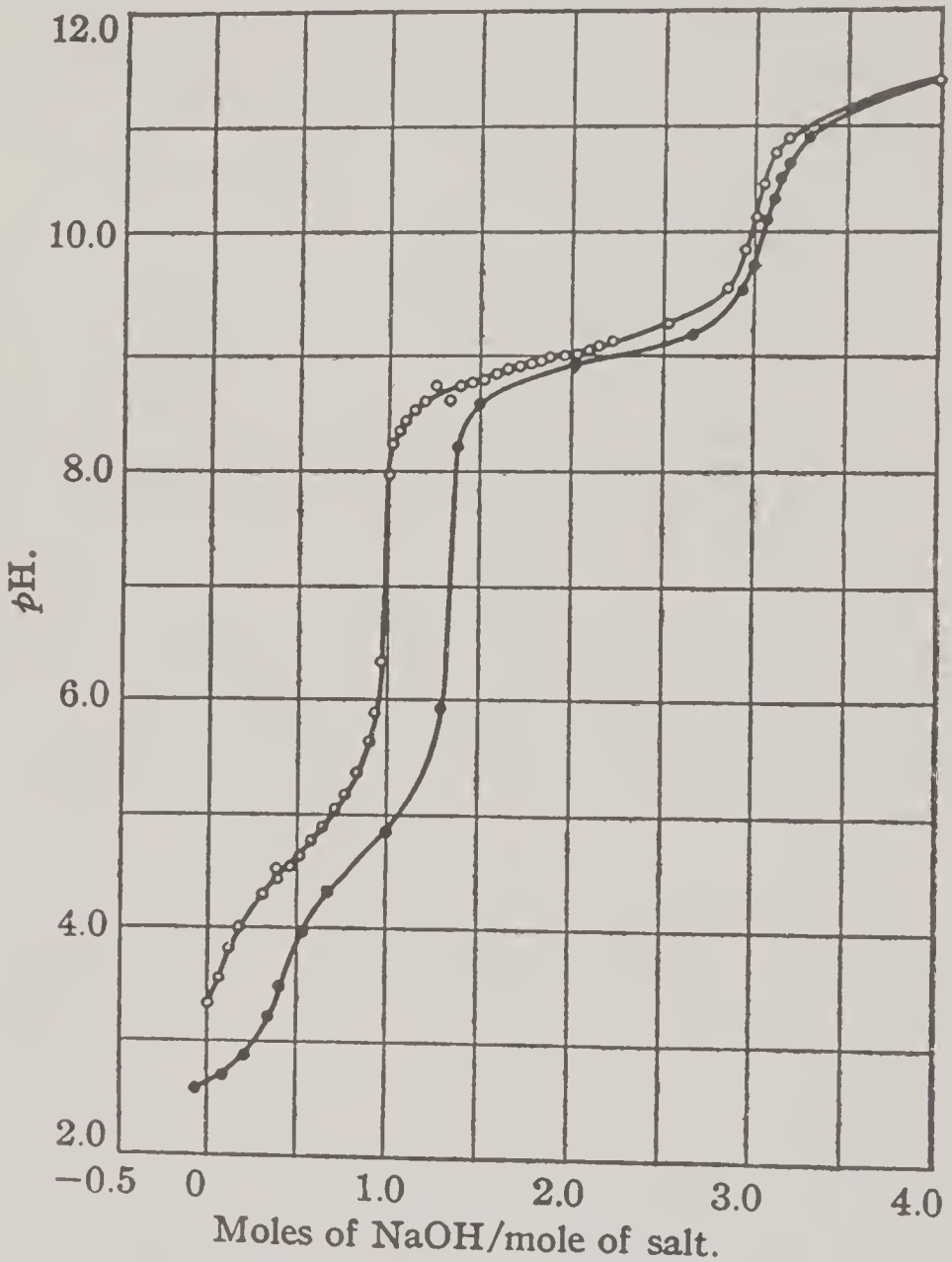
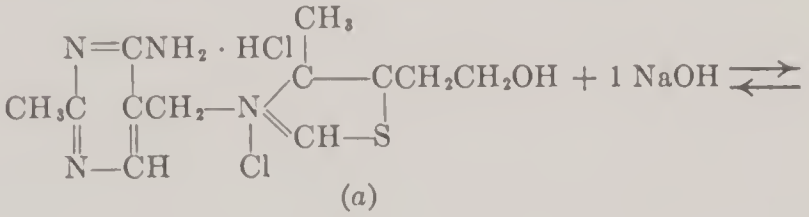


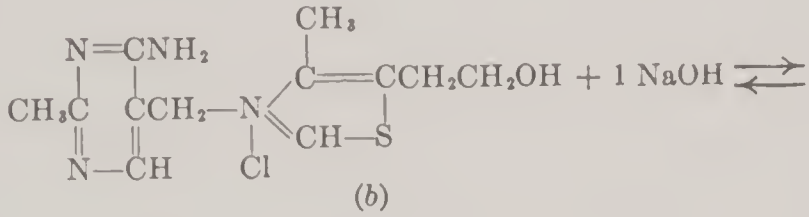
FIGURE 6. Titration of vitamin B_1 chloride hydrochloride: o—o titration with NaOH, •—• back titration with HCl. Courtesy of *Journal American Chemical Society*.

addition of 1 mole of alkali liberates the monochloride (*b*), see below; further addition of alkali begins to liberate the strong quaternary base (*c*) which goes over rapidly into the neutral pseudo base or carbinol (*d*). This does not occur instantaneously, as one can see during titration by the momentary rise and subsequent slow fall of *p*H after each addition of alkali. Such process of the migration of hydroxyl is well known in cyclic quaternary bases and in effect uses up hydroxyl ions. Nor does the *p*H rise

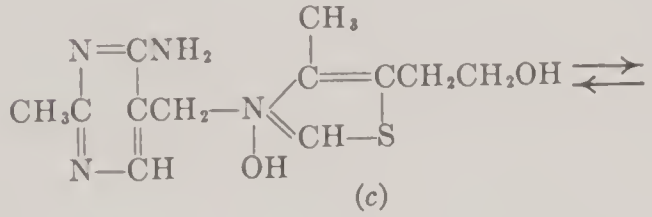
even when 2 moles have been added, because quaternary thiazoles such as this undergo ring opening in alkaline solution, forming an acidic sulfhydryl group (22). Only after this is neutralized with a third mole of alkali to form (*e*) does the alkalinity rise sharply, indicating free NaOH. The reverse arrows indicate reversal of the sequence and the regeneration of the vitamin upon back titration. If the solution stands in alkaline condition for some time, there is some permanent destruction of the vitamin.



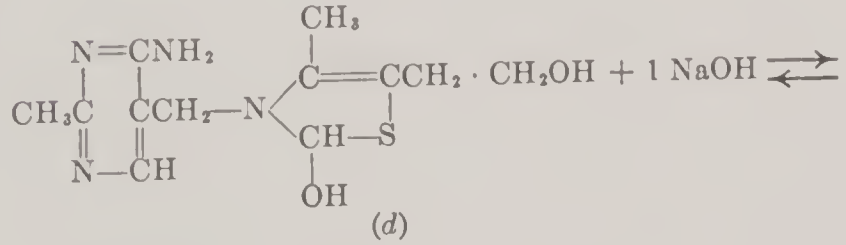
(*a*)



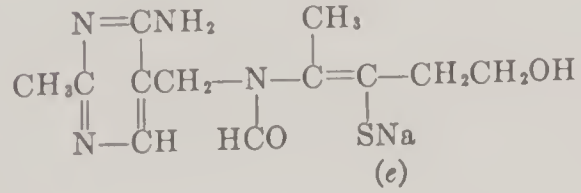
(*b*)



(*c*)



(*d*)



(*e*)

Sensitivity to *p*H

The changes which take place in alkaline solution may have played some role in the arduous task of isolating thiamin. However, prolonged exposures under neutral conditions probably were of greater importance as a destructive agency than strongly alkaline conditions which had to be maintained for only relatively short periods of time.

This view is based in part on the consideration that the sul-

fite cleavage cannot in all probability be an absolutely unique reaction. The odor of the thiazole portion of the molecule is present during all the later stages of isolation of thiamin from natural sources, and it is by no means unreasonable to suppose that cleavage may be promoted by the presence or absence of other substances. As a matter of direct observation the sensitivity of the vitamin to *p*H appears to depend greatly on the nature of the medium.

An experience encountered during isolation is worthy of record here. A large quantity of quinine sulfate eluate of activated fuller's earth was largely freed of quinine by neutralizing with baryta. It was then brought to *p*H 4.5 with sulfuric acid and divided into two portions. One of these was evaporated *in vacuo* at $< 35°$ with a loss of 50 per cent of the activity. The other portion after standing in a closed vessel for six months showed no loss of activity. After acidifying to *p*H 3.5, it was evaporated *in vacuo* also without loss of activity. This experience suggests a reaction involving the loss of formaldehyde during evaporation. Kircher (23) has disclosed such a loss of formaldehyde in a 5-methylol uracil. However, such a reaction of thiamin has not been successfully demonstrated. At all events there appears to be a relatively feeble union of the two nuclei under neutral conditions. Hot alkali splits out the sulfur from thiamin as H_2S but does not disrupt the molecule generally (24, 25).

Other Reactions

Thiamin is converted to chloroxy vitamin by the action of strong hydrochloric acid (page 175). Nitrous acid under mild conditions does not affect it (26), but in the presence of free mineral acid with heat, nitrogen is evolved, perhaps with the formation of the corresponding hydroxy compound. The vitamin resists the action of benzoyl chloride under Schotten Baumann conditions. It may be acetylated without great loss of activity, reaction presumably occurring on the alcoholic side chain of the thiazole. Acetic anhydride at high temperature inactivates it. Formaldehyde is said to destroy it (27), as do ultraviolet light (28) and X rays (29).

The vitamin gives a number of color reactions. The earliest of these is the formaldehyde azo test (30). Diazotized sulfanilic acid is treated with sodium carbonate and mixed with the vitamin solution to which a drop of formaldehyde has just previously been added. A yellow color develops which becomes pink in ten minutes and persists for days. Reducing substances interfere. Similarly diazotized paraamino acetanilide or methylparaaminoacetophenone couple with thiamin to produce red-purple com-

pounds insoluble in water but soluble in immiscible solvents (31). Another color test utilizing p-dimethylamino benzaldehyde and acetic acid is reported by Tauber (32). In the presence of thiamin an intense brick-red color is produced which is believed to be due to a Schiff's base.

Thiamin gives colored precipitates with picrolonic acid, gold or mercuric chloride, Mayer's reagent, and iodine. Potassium bismuth iodide gives a characteristic orange-red precipitate (33). Crystalline precipitates are yielded by potassium tetranitrodiamino colbaltate rufianic acid and by Reinecke's salt. Amorphous salts are precipitated by picric acid (from relatively concentrated solutions), alizarinsulfonic acid, β anthraquinone sulfonic acid, trinitroresorcinol, potassium periodide, and perbromide (34). In fact, a very wide variety of alkaloidal reagents precipitate it. Platinic chloride precipitates it only from absolute alcohol.

Simple Salts

The most familiar form of thiamin is the chloride hydrochloride in the form of acicular crystals m.p. 248°–250° with decomposition. However, this salt also crystallizes under certain conditions with m.p. 232°–234°, a phenomenon for which there is no explanation in terms of structural chemistry (35, 36, 37). The picrolonate also has been isolated in two forms of different melting points but of identical composition, m.p. 165° and 229°, respectively. These salts have the same physiological activity. This is also true of the two crystal forms of the chloride hydrochloride. Their ultraviolet absorptions are also identical. The characteristic curve is shown in Figure 5. There are several cases of discrepancies in the melting points of pyrimidine intermediates which have been prepared in this connection. These may possibly be due to the same phenomenon.

Two sulfates have been described, but these on the basis of somewhat unsatisfactory analyses appear to have different compositions corresponding to $2x(SO_4)_3$ and $2x(SO_4)_4$, where x represents a mole of the base. Their melting points are 203° and 276–8°, respectively. The nitrate decomposes sharply at 164°–165° (38). The bromide hydrobromide hemihydrate melts at 229°–231°. The chloride hydrochloride also crystallizes from alcohol water solution as a hemihydrate. However, the proportion of water of crystallization, if it can be properly so called, is not constant over the usual range of atmospheric humidities varying from about 1 mole to a nearly anhydrous condition. This feature was very troublesome in establishing the correct empirical formula of the substance as isolated from natural sources.

The free base has never been obtained. It is undoubtedly extremely unstable.

Tests for Identity and Purity

The commercial product, crystalline vitamin B_1, is a pure chemical compound, $C_{12}H_{17}ON_4SCl \cdot HCl$, containing approximately 1 mole of water of crystallization, melting at about 245° with decomposition and leaving no ash on ignition. It occurs in the form of colorless or white, odorless crystals, or as a crystalline powder with a slightly saline taste. It is very soluble in water and very slightly soluble in absolute alcohol. Its aqueous solution (1:20) is acid to litmus (*p*H about 2.5) and is optically inactive.

Crystalline vitamin B_1 is tested for identity and purity as follows:

A 1 per cent aqueous solution of crystalline vitamin B_1 yields a yellow precipitate with picric acid T.S. (T.S. refers to the test solutions of the U. S. Pharmacopoeia); a white precipitate with mercuric chloride T.S.; a yellowish-white precipitate with Mayer's reagent; and a red-brown precipitate with iodine T.S.

Dissolve 5 mg. of crystalline vitamin B_1 in a mixture of 1 cc. of lead acetate T.S. and 1 cc. of 10 per cent sodium hydroxide solution. A yellow color is produced. On heating the mixture for several minutes on the steam bath the color changes to brown, and on standing, lead sulfide precipitates.

Prepare two solutions as follows: Solution A. Place 1.5 cc. of a 5 per cent sodium nitrite solution in a volumetric flask immersed in an ice bath, and add 1.5 cc. of sulfanilic acid solution (prepared by dissolving 0.9 gram sulfanilic acid in 9 cc. of hydrochloric acid and diluting to 100 cc. with water). Keep in the ice bath for 5 minutes, then add an additional 6 cc. of the sodium nitrite solution, mix thoroughly, and allow to stand in the ice for 5 minutes longer. Dilute to 50 cc. with cold water, mix and cool in the ice for 15 minutes. Solution B. Dissolve 1.15 grams of sodium bicarbonate in 20 cc. of water and add 20 cc. of sodium hydroxide T.S. Add 1.5 cc. of Solution B to 0.5 cc. of solution A and after one minute add 1 drop of formaldehyde and then immediately add 1 cc. of a solution of 5 mg. of crystalline vitamin B_1 in 50 cc. of water. A pink color develops slowly and increases in intensity on standing.

An aqueous solution of crystalline vitamin B_1 (1:100) yields a white precipitate with silver nitrate T.S., which is insoluble in nitric acid but soluble in ammonia water.

Moisten 0.1 gram of crystalline vitamin B_1 with 5 drops of sulfuric acid and ignite. No weighable residue remains.

To 5 cc. of a 2 per cent aqueous solution of crystalline vita-

min B_1 add 5 cc. of sodium hydroxide T.S. and heat gently. The escaping vapors do not turn moistened red litmus paper blue (ammonium salts).

To a solution of 0.1 gram crystalline vitamin B_1 in 10 cc. of water, add 2 drops of dilute hydrochloric acid and 10 cc. of hydrogen sulfide T.S. No darkening is produced (heavy metals).

To a solution of 0.1 gram crystalline vitamin B_1 in 10 cc. of water, add 1 cc. of dilute hydrochloric acid and 1 cc. of barium chloride T.S. No turbidity should develop in 5 minutes (sulfate).

Drying. Weigh accurately about 0.5 gram and dry in a vacuum desiccator over sulfuric acid for twenty-four hours. The loss in weight corresponds to not more than 5 per cent (water).

Nitrogen. Determine the nitrogen on a portion of the sample obtained from the previous test by the Kjeldahl-Gunning-Arnold method, or micro-analytically by the Dumas method. It shows not less than 16.3 per cent and not more than 16.8 per cent of nitrogen (theory, 16.62 per cent).

Sulfur. Weigh accurately about 0.3 gram of the dried sample from the test for water and explode it with sodium peroxide, potassium perchlorate, etc., in a Parr Bomb, using the standard quantities of the reagents, and follow the directions prescribed for the Parr Bomb explosions. Treat the mass with 150 cc. of water, add just sufficient hydrochloric acid to neutralize, and add 1 cc. in excess. Heat to boiling, filter if necessary, wash and precipitate the hot filtrate with barium chloride solution. Digest on the steam bath for several hours or allow to stand over night, wash a few times by decantation with water, filter, then wash until the washings cease to give a reaction for chloride, dry, and ignite. The weight of the barium sulfate, after correcting for any sulfate derived from the reagents, corresponds to not less than 9.4 per cent and not more than 9.8 per cent sulfur (theory, 9.50 per cent).

Units

The widely used units of vitamin B_1 are somewhat uncertain quantities (see Chapter XVII). For rigorous scientific purposes the International Unit cannot be assigned a fixed value, as the standard adsorbate varies in effectiveness according to the type of test used. However, for the use of non-specialists some simplification is necessary even at the expense of precision. The following table gives figures which are now widely accepted by the drug trade in America and represent a practical compromise. In accordance with it most American drug firms marketing the synthetic vitamin rate on their labels 1 International Unit as equal to 3.33 γ.

TABLE OF APPROXIMATE EQUIVALENTS

1 gram vitamin B_1 crystals (assayed by modified Smith rat curative technique)	300,000 International Units 150,000 Smith Curative Units 600,000 Chase-Sherman Units 300,000 Roscoe Units
1 International Unit	0.5 Smith Curative Unit 2.0 Chase-Sherman Units 1.0 Roscoe Unit 20.0 milligram-equivalents (Cowgill) 3.33 micrograms vitamin B_1 crystals
1 Smith Unit (modified rat curative technique)	2.0 International Units 4.0 Chase-Sherman Units 6.67 micrograms vitamin B_1 crystals
1 milligram-equivalent (Cowgill)	0.05 International Unit

In explanation of the Smith Unit in the table it may be said that Smith originally proposed a unit consisting of the quantity required to cure every polyneuritic rat in a series used for tests. 6.7 micrograms (γ) of crystalline thiamin will approximate this result. Many workers, however, have followed the modified standard of Waterman and Ammerman, which called for the cure of 70 per cent of the polyneuritic animals. The quantity necessary to do this is about 5 γ.

In Continental Europe and to some extent in England, the International Unit is often taken as 2 γ of thiamin. Some, however, use 3 γ. There is, therefore, great need for agreement. The International Conference on Vitamin Standardization is expected to hold a session during 1938. A standard lot of the crystalline substance has been widely distributed to various laboratories in Europe and America for thorough testing, the results to be discussed at the Conference.

The Conference Committee has expressed the hope that a new standard, based on the crystalline material, can be adopted. There is much reason for believing that the public welfare would be best served by abandoning the use of any special unit for vitamin B_1 and encouraging the expression of all assay values in terms of weight of crystalline substance. Special arbitrary units are required only for therapeutic agents, the effective principle of which cannot be isolated, identified, and weighed.

BIBLIOGRAPHY

1. EIJKMAN, C.: Über Ernährungspolyneuritis. Arch. Hyg. *58*, 150, 1906.
2. CHICK, H., and HUME, E. M.: The Effect of Exposure to Temperatures at or above 100° C. upon the Substance (Vitamin) Whose Deficiency in a Diet Causes Polyneuritis in Birds and Beriberi in Man. Proc. Roy. Soc. *B90*, 60, 1917.

3. Holst, A.: Experimental Studies Relating to Ship Beriberi and Scurvy. Jour. Hyg. *7*, 634, 1907.
4. Vedder, E. B.: Is the Neuritis Preventing Vitamin Concerned in Carbohydrate Metabolism? Jour. Hyg. *17*, 1, 1918.
5. Sherman, H. C., and Grose, M. R.: A Quantitative Study of the Destruction of Vitamin B by Heat. Jour. Amer. Chem. Soc. *45*, 2728, 1923.
6. Sherman, H. C., and Burton, G. W.: Effect of Hydrogen-ion Concentration on the Rate of Destruction of Vitamin B. Jour. Biol. Chem. *70*, 639, 1926.
7. Halliday, N.: Effect of Heat at Varying Concentrations of Hydrogen-ion on Vitamin B (B_1) in Protein Free Milk. Jour. Biol. Chem. *98*, 707, 1932.
8. Keenan, J. A., Kline, O. L., Elvehjem, C. A., and Hart, E. B.: Stability of Vitamin B (B_1), G (B_2) and B_4. Jour. Nutrition *9*, 63, 1935.
9. Guha, B. C., and Drummond, J. C.: Observations on the Concentration of Vitamin B_1. Biochem. J. *23*, 880, 1929.
10. Baker, A. Z., and Wright, M. D.: Vitamin B_1 in Human Diets. Proc. Roy. Soc. Med. *29*, 1145, 1936.
11. Ohdake, S., and Yamagishi, T.: Oryzanin, "Antineuritic Vitamin." On the Activity and Thermostability of Oryzanin Hydrochloride. Bull. Agric. Chem. Soc. Japan *11*, 51, 1935.
12. Barger, G., Bergel, F., and Todd, A. R.: Crystalline Fluorescent Dehydrogenation Products from Vitamin B_1. Nature *136*, 259, 1935.
13. Williams, R. R., Waterman, R. E., Keresztesy, J. C., and Buchman, E. R.: Studies of Crystalline Vitamin B_1. III. Cleavage of Vitamin with Sulfite. Jour. Amer. Chem. Soc. *57*, 536, 1935.
14. Morgan, A. F.: Nutritive Value of Dried Fruits. Amer. Jour. Pub. Health, *25*, 328, 1935.
15. Van Veen, A. G., and Koks, M. T.: Effect of the Clayton Process on the Vitamin B_1 Content of Rice. Geneeskund. Tijdschr. Ned. Ind. *74*, 482, 1934.
16. Lipmann, F.: Hydrogenation of Vitamin B_1. Nature *138*, 1097, 1936.
17. Warburg, O., and Christian, W.: Verbrennung von Robisonester durch Triphosphopyridinnucleotid. Biochem. Z. *287*, 440, 1936.
18. Karrer, P., and Warburg, O.: Jodmethylat des Nicotinsäureamids. Biochem. Z. *285*, 297, 1936.
19. Lipmann, F.: A Coloured Intermediate on Reduction of Vitamin B_1. Nature *140*, 849, 1937.
20. Michaelis, L.: The Formation of Semiquinones as Intermediary Reduction Products from Pyocyanine and Some Other Dyestuffs. J. Biol. Chem. *92*, 211, 1931.
21. Cline, J. K., Williams, R. R., Ruehle, A. E., and Waterman,

R. E.: Studies of Crystalline Vitamin B_1. XVI. Identification of the Pyrimidine Portion. Jour. Amer. Chem. Soc. *59,* 530, 1937.

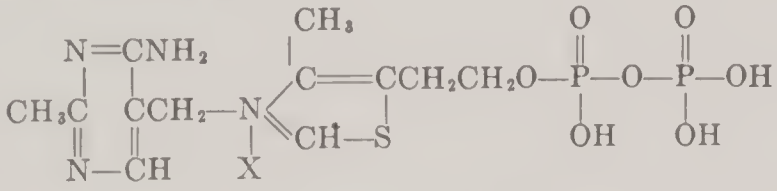
22. Mills, W. H., Clark, L. M., and Aeschlimann, J. A.: Studies in the Benzothiazole Series. Part I. The Pseudo-bases of the Benzothiazole Quaternary Salts. Jour. Chem. Soc. *123,* 2353, 1923.
23. Kircher, A.: Über die Kondensation von Methyluracil mit Formaldehyd. Ann. Chem. *385,* 293, 1911.
24. Clarke, H. T., and Gurin, S.: Studies of Crystalline Vitamin B_1. XII. The Sulfur Containing Moiety. Jour. Amer. Chem. Soc. *57,* 1876, 1935.
25. Kinnersley, H. W., O'Brien, J. R., and Peters, R. A.: The Properties of Blue Fluorescent Substances Formed by the Oxidation of Vitamin B_1 (Quinochromes). Biochem. J. *29,* 2369, 1935.
26. Peters, R. A.: The Action of Nitrous Acid upon the Antineuritic Substances in Yeast. Biochem. J. *18,* 858, 1924.
27. Malcolm, J.: Effect of Formaldehyde on a Vitamin B_1 Preparation (Tiki-tiki). Quart. Jour. Exp. Physiol. *23,* 83, 1933.
28. Heyroth, F. F., and Loofbourow.: Irradiation of Yeast Oryzanin. Jour. Amer. Chem. Soc. *56,* 2010, 1934.
29. Sugiura, K.: The Effect of X-rays on the Vitamin B Content of Wheat Seedlings. Radiology *21,* 438, 1933.
30. Kinnersley, H. W., and Peters, R. A.: The Formaldehyde-azo Test for Vitamin B_1. Biochem. J. *28,* 667, 1934.
31. Prebluda, H. J, and McCollum, E. V.: A Chemical Reagent for the Detection and Estimation of Vitamin B_1. Science *84,* 488, 1936.
32. Tauber, H.: A Color Test for Thiamin (Vitamin B_1). Science *86,* 594, 1937.
33. Naiman, B.: A Reagent for Vitamin B_1. Science *85,* 290, 1937.
34. Rosenthaler, L.: Über zwei Kristallfällungen des Vitamins B_1. Zeit. Vitaminforsch. *5,* 276, 1936.
35. Williams, R. R., and Cline, J. K.: Synthetic Vitamin B_1. Jour. Amer. Chem. Soc. *59,* 216, 1937.
36. Todd, A. R., and Bergel, F.: Aneurin. Part VII. A Synthesis of Aneurin. Jour. Chem. Soc., p. 364, 1937.
37. Bergel, F., and Todd, A. R.: Aneurin. Part IX. A New Synthesis of Thiochrome. Note on the Synthesis of Aneurin. Jour. Chem. Soc., p. 26, 1938.
38. Kinnersley, H. W., O'Brien, J. R., and Peters, R. A.: Crystalline Vitamin B_1. Biochem. J. *29,* 701, 1935.

CHAPTER XIV

FUNCTIONAL GROUPS OF THE THIAMIN MOLECULE

One could be pardoned for hoping that a knowledge of the chemical structure of thiamin would promptly suggest the mode of its action and resolve many of the problems that have plagued us for a generation. The natural processes into which it enters are, however, so complicated that for the present at least this knowledge only opens up a vista of new problems. The progress of the past year has been such as to justify optimism for the future, but the gratification of our more sanguine hopes must be deferred for a time.

Probably the most important single advance in thiamin chemistry during 1937 was the isolation of the so-called cocarboxylase (1–4). It is depicted as the pyrophosphoric ester of thiamin having the structure:



Like the vitamin itself, it is split by sulfite to yield the identical pyrimidylmethylsulfonic acid as well as the same thiazole but in pyrophosphorylated form. It possesses antineuritic activity very similar to that of the vitamin, but no details on this phase of the matter have been published. It is, therefore, impossible to state precisely how it compares with free thiamin in this regard. Like thiamin the pyrophosphate is oxidized by ferricyanide to a thiochrome-like substance. It also functions to enhance the oxygen uptake of polyneuritic brain tissue in a pyruvate phosphate medium, but according to Peters only to about one tenth the extent that the free vitamin does (5). However, Lohmann and Schuster report it equally active in this respect. One phosphoric acid molecule is removed by hydrolysis with aqueous acid very readily, the second with difficulty.

The pyrophosphate may be distinguished from free thiamin by the fact that with washed yeast cells in pyruvate medium it causes the evolution of carbon dioxide according to the equation:

$$CH_3COCOOH \longrightarrow CH_3CHO + CO_2$$

It stimulates the growth of microorganisms as thiamin does, and there is a great deal to suggest that, in many instances at least, the biological utilization of thiamin is preceded by its conversion to the pyrophosphate. The pyrophosphate of thiamin is regarded as the prosthetic group of an enzyme or enzymes, each of which comprises a specific protein component also. In the above example the washed yeast cells supply the protein component. Neither the thiamin pyrophosphate nor the protein is effective by itself, but the mixture of the two is operative. A strong parallelism is, therefore, set up between vitamin B_1 and vitamin B_2 (riboflavin) (see page 132), both functioning as coenzymes. The coenzyme function of thiamin is discussed at length in Chapter XXVI.

The cocarboxylase as isolated from yeast is a crystalline substance. It is said to occur in yeast in an amount which corresponds very well with the thiamin content of yeast. The statement has also been made, apparently with equally good evidence, that thiamin occurs largely as the pyrophosphate in yeast and in the free form in rice polish. It has been suggested that the two forms of vitamin may occur in dynamic equilibrium in animal tissue (6). The synthesis of the pyrophosphate in amounts sufficient for biological test but not for isolation of the pure substance has been effected by the action of $POCl_3$ on free thiamin (7). An enzymatic synthesis from thiamin by the action of washed yeast cells and by duodenal mucosa of the pig gives nearly 100 per cent conversion (8). Other enzymatic syntheses have been reported (9) and the mechanism of the phosphorylation of thiamin has been somewhat studied but is not yet clear. Hexose diphosphate, adenosine triphosphate, and phosphopyruvic acids have been considered as phosphate donators in the reaction. Iodoacetic acid inhibits the enzymatic synthesis but fluoride does not (6).

Whether or not the pyrophosphate is the sole or principal form of thiamin which functions in the animal body is questionable. There is much to suggest that it is the coenzyme for more than one enzymatic system, and free thiamin or perhaps thiamin in some other combination may also function in some way. As far as formation of the pyrophosphate is concerned, however, it would seem that the alcohol group of the thiazole side chain is essential. There is much to indicate that this group is necessary for any form of biological activity. Thus Cline has found (unpublished results) that the ether of thiamin is inactive upon polyneuritic animals, while its esters are active. The ethers do not hydrolyze readily to free the hydroxyl group, while the esters do. The structure of those thiazoles which are active for some plants and microorganisms also points in the same direction (see Chapter XVI).

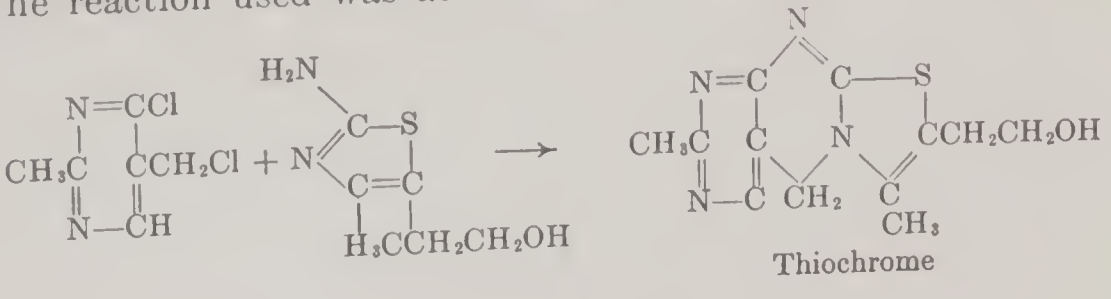
Thiochrome

The amino group on the pyrimidine ring is also essential for antineuritic activity as shown by similar evidence. Thiochrome represents the earliest example of a substance closely related to thiamin chemically, which is, however, physiologically inactive for animals and largely so for plants and microorganisms.

This very interesting substance is a yellow crystalline sulfur containing pigment derived from yeast (10–13). It has the composition $C_{12}H_{14}N_4OS$, closely resembling the theoretical free base of thiamin, $C_{12}H_{17}ON_4S \cdot OH$, and differing from it only by the subtraction of a mole of water and two hydrogen atoms. A structural resemblance between the two compounds was early suspected. Barger and associates (14, 15) showed that thiamin could be oxidized to thiochrome very readily under mild conditions, as by the use of alkaline ferricyanide. Other oxidizing agents may be used (16) but ferricyanide has remained the preferred reagent for the purpose. This reaction has been tried rather extensively and with fair success for the chemical determination of thiamin (see page 217). Thiochrome is recognized and measured by its intense blue fluorescence in daylight.

The study of its structure proceeded in parallel with the elucidation of that of thiamin and contributed much to the latter. While the vitamin loses an amino group by hydrolysis, thiochrome does not do so. Further, the fact of blue fluorescence suggested a three-ring system in thiochrome. The central and distinctive ring was early supposed to be formed upon the nitrogen atom of the amino group of thiamin. This was the basis of the earliest formulation of a structure for thiochrome by Barger and associates, and of a later formulation by Makino and Imai (17). Other early evidence of the contrast between thiamin and thiochrome was found in the positive Peters' formaldehyde azo test of thiamin, which is negative in the case of thiochrome. This was for some time attributed to the free amino group on the pyrimidine, but has latterly been ascribed to the thiazole portion of the molecule (see below).

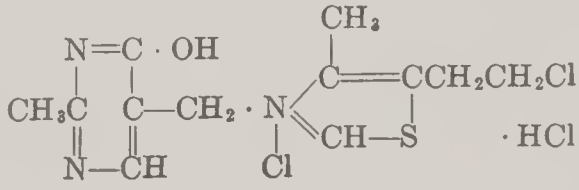
As soon as the structure of thiamin was correctly formulated (18), that of thiochrome promptly followed (19) and this was presently confirmed by synthesis by Todd and associates (20). The reaction used was as follows:



Thiochrome

Probably those who first worked with thiochrome cherished the thought that it would prove to be reversibly reducible to thiamin and thus represent a stage in the catalytic utilization of the vitamin in organisms. If so, they fortunately exercised an excellent restraint in avoiding a premature statement of opinion, for later events have brought out nothing to substantiate such a view. Thiochrome now appears to be a degradation product of vitamin which has already passed its day of usefulness. However, this statement must also be made with reservation.

Another derivative of thiamin which threw light upon the importance of the amino group in relation to the physiological activity of thiamin is the so-called chloroxy vitamin (21). It has the structure:



possesses no physiological activity, and yields no thiochrome-like product on oxidation with thiochrome, although its ultraviolet absorption strongly resembles that of thiamin. Later experience has shown that the thiochrome reaction is an excellent test for the presence of the amino group in analogues of thiamin. Oxy vitamin, which differs from chloroxy vitamin only in the replacement of the nonionic chlorine by hydroxyl, is also inactive physiologically and does not give the thiochrome reaction.

A study of several analogues of thiamin has been published (22) which affords further evidence as to the groups which are functionally important. According to these results an amino group on the pyrimidine, a 5-β-hydroxyethyl group on the thiazole, a hydrogen atom in position 2 of the thiazole, and a methylene bridge between thiazole and pyrimidine are all indicated as necessary to physiological activity. The formaldehyde azo test is given by those analogues containing a 5-β-hydroxyethyl group and a hydrogen in position 2 of the thiazole; the thiochrome test by those with an amino group in position 4 (or 6) of the pyrimidine, and a hydrogen in position 2 of the thiazole. J. K. Cline (unpublished results) has found that replacement of one or both of the hydrogens of the amino group of the pyrimidine by alkyl groups results in a hundredfold reduction of the antineuritic curative action but does not destroy it altogether. The thiochrome test for such compounds is negative, as one would expect.

Thiochrome and an analogue of thiamin which lacks the ethyl alcohol side chain on the thiazole were found nearly or quite inactive in promoting the growth of staphylococcus un-

less supplemented by the thiazole of the proper structure (23). Evidently the organism can extract the pyrimidine from these analogues and utilize it in conjunction with the proper thiazole if the latter is separately supplied. (See Chapter XXVIII for details on the behavior of microorganisms.)

A different complexion is given to the matter of physiologically functional groups in the thiamin molecule by German publications (24), which depict a whole series of compounds of physiological activity resulting from the substitution of various groups about the molecule. This view is also expressed in the patent literature (25). In varying degree these communications indicate a great latitude of choice of groups about the molecule. Particular emphasis is placed upon the possibility of increasing the size of the alkyl group upon the pyrimidine and lengthening the bridge. Since the publications are not consistent among themselves and are not explicit as to the organisms used, the type of tests or the dosages required, we refrain from a statement about physiological activity of these variants pending publication of details. Suffice it to say that some degree of activity for plants or microorganisms is no indication of activity for animals as will be seen in the next following chapters. Further, a curative action on pigeons or even rats cannot be fully trusted as evidence of complete physiological equivalence to the vitamin. Pigeons often show spontaneous cures or respond in curative tests to substances which are not able to protect the birds over periods of weeks from the consequences of vitamin B_1 deficiency. Only protective tests on animals can be taken as final evidence. The effects must include the favorable influence on appetite, growth, utilization of food, etc., which characterize the substance of natural origin. For the present it is safest to suppose on the grounds of biological adaptation that thiamin is a unique or nearly unique molecule which cannot undergo alteration without impairment of physiological utility.

A list of thiamin analogues follows in summary form convenient for reference. Only those are included for which chemical criteria have been established.

Analogues of Thiamin

1. $C_9H_{11}ClN_3O_2S$ 3-(2′,6′-dihydroxy-4′-methylpyrimidyl-5′)-4-methylthiazolium chloride, m. 306° (decompn.). From 5-thioformamido-4-methyluracil and chloracetone (26).
2. $C_9H_{12}Cl_2N_4S$ 3-(6′-amino-4′-methylpyrimidyl-5′)-4-methylthiazolium chloride hydrochloride, m. 254–5° (decompn.). From 6-amino-5-thioformamido-4-methylpyrimidine chloracetone and C_2H_5ONa (27). Fluorescent in ultraviolet light when oxidized with alk. $K_3Fe(CN)_6$. Inactive for pigeons in 5 mg. doses.

3. $C_9H_{13}Cl_2N_5S \cdot 3\,H_2O$ 3-(2′,6′-diamino-4-methylpyrimidyl-5′)-4-methylthiazolium chloride hydrochloride, m. 315° (decompn.); picrate, m. 255°. From 2,6-diamino-5-thioformamido-4-methylpyrimidine and chloracetone (26). Blue fluorescence in ultraviolet light (not in visible) when treated with alk. $K_3Fe(CN)_6$.
4. $C_{10}H_{12}ClN_3OS \cdot HCl$ 3-(4′-hydroxy-2′-methylpyrimidyl-5′-methyl)-4′-methylthiazolium chloride hydrochloride, m. softens 220°. From 4-hydroxy-5-thioformamidomethyl-2-methylpyrimidine and chloracetone (22). Thiochrome test negative. Inactive for pigeons in 8 mg. doses.
5. $C_{10}H_{13}ClN_4S \cdot HCl$ 3-(4′-amino-2′-methylpyrimidyl-5′-methyl)-4-methylthiazolium chloride hydrochloride, m. blackens at 261–2°. From 4-amino-5-thioformamidomethyl-2-methylpyrimidine and chloracetone (22). Thiochrome test positive. Inactive for pigeons.
6. $C_{10}H_{14}Cl_2N_4S$ 3-(6′-amino-4′-ethylpyrimidyl-5′)-4-methylthiazolium chloride hydrochloride, m. 252–3° (decompn.). From 6-amino-5-thioformamido-4-ethylpyrimidine and chloracetone and C_2H_5ONa heated 3 hrs. (27). Weak fluorescence in ultraviolet light when oxidized with alk. $K_3Fe(CN)_6$. Inactive for pigeons in 2.8 mg. doses.
7. $C_{12}H_{14}N_4OS$ Thiochrome, see p. 174, m. 225–6°. From 4-chloro-5-chloromethyl-2-methylpyrimidine and 2-amino-4-methyl-5-(β-hydroxyethyl)thiazole heated at 110° for 15 min. (20), m. 221°; sublimes at 210-5° *in vacuo;* hydrochloride, m. above 200° (decompn.). From the vitamin in CH_3OH and KOH and $K_3Fe(CN)_6$. (15). Formaldehyde-azo test negative. No antineuritic activity. Intense blue fluorescence only in neutral or alkaline solution (14).
8. $C_{12}H_{14}Cl_3N_3OS$ 3-(2′,4′-dichloro-6′-methylpyrimidyl-5′-methyl)-4-methyl-(5-β-hydroxyethyl)thiazolium chloride, m. 206°; picrate, m. 142°. From 2,4-dichloro-5-chloromethyl-6-methylpyrimidine and 4-methyl-(5-β-hydroxyethyl)thiazole (22). Thiochrome test negative.
9. $C_{12}H_{14}Cl_2IN_3OS$ 3-(2′,4′-dichloro-6-methylpyrimidyl-5′-methyl)-4-methyl-(5-β-hydroxyethyl)thiazolium iodide, m. 181–2°; picrate, m. 142°. From 2,4-dichloro-5-iodomethyl-6-methylpyrimidine and 4-methyl-(5-β-hydroxyethyl)thiazole (22).
10. $C_{12}H_{16}Cl_3N_3OS$ Chloroxy vitamin; from the vitamin and conc. HCl. 3-(2-methyl-4-hydroxypyrimidyl-5-methyl)-4-methyl-5-chlorethylthiazolium chloride hydrochloride (21). No activity for rats. Thiochrome test with $K_3Fe(CN)_6$ negative.
11. $C_{12}H_{16}Cl_2N_4OS$ 3-[(2′-chloro-4′-amino-6′-methylpyrimidyl-5′-methyl)-4-methyl-(5-β-hydroxyethyl)]thiazolium chloride, m. 200-5°; picrate, m. 214–5°. From 3-[(2′,4′-dichloro-6′-methylpyrimidyl-5-methyl)-4-methyl-(5-β-hydroxyethyl)]thiazolium chloride and alc. NH_3 (22). Thiochrome test positive. Inactive for pigeons in 0.5 and 1.0 mg. doses. Corresponding iodide similarly prepared, m. 228° (decompn.). No activity.

12. $C_{12}H_{16}ClN_3O_2S \cdot HCl$ 3-[(4′-hydroxy-2′-methylpyrimidyl-5′-methyl)-4-methyl-(5-β-hydroxyethyl)]thiazolium chloride hydrochloride, m. froths 170°, melts completely at 195–7°. From 4-hydroxy-5-thioformamidomethyl-2-methylpyrimidine and methyl-α-bromo-γ-acetoxypropyl ketone (22). Thiochrome test negative. Inactive in 1 mg. dose.
13. $C_{12}H_{18}Cl_2N_4OS$ 4-methyl-5-hydroxyethyl-N-[(4-amino-6-methyl-pyrimidyl-5-methyl)]thiazolium chloride hydrochloride, m. 242°; picrolonate, m. 213°. From 4-amino-5-bromomethyl-6-methyl-pyrimidine hydrobromide and 4-methyl-5-hydroxyethylthiazole (24). Gives a thiochrome-like product on oxidation with $K_3Fe(CN)_6$.
14. $C_{12}H_{18}Cl_2N_4OS$ 3-(6′-amino-4′-ethylpyrimidyl-5′)-4-methyl-(5-β-hydroxyethyl)thiazolium chloride hydrochloride, m. 220° (decompn.). From 6-amino-5-thioformamido-4-ethylpyrimidine and methyl-α-chlorohydroxypropyl ketone and C_2H_5ONa (26). Fluorescent in ultraviolet light (not in visible) when oxidized with alk. $K_3Fe(CN)_6$. Inactive for pigeons in 1.2 mg. dose.
15. $C_{13}H_{19}ClN_4OS \cdot HCl \cdot H_2O$ 3-[(4′-amino-2′-methylpyrimidyl-5′-methyl)-2,4-dimethyl-(5-β-hydroxyethyl)]thiazolium chloride hydrochloride. From No. 16 below by treatment with HCl (22), m. 199°; picrate, m. 218–9°. Thiochrome test negative. Inactive for pigeons in 100 γ doses.
16. $C_{15}H_{21}BrN_4O_2S \cdot HBr \cdot H_2O$ 3-[(4′-amino-2′-methylpyrimidyl-5′-methyl)-2,4-dimethyl-(5-β-acetoxyethyl)]thiazolium bromide hydrobromide, "Methylaneurin," m. 193–4°; picrate, m. 188–9°. From 4-amino-5-thioacetamidomethyl-2-methylpyrimidine and methyl-α-bromo-γ-acetoxypropyl ketone (22).

BIBLIOGRAPHY

1. Lohmann, K., and Schuster, Ph.: Über die Cocarboxylase. Naturwissensch. *25*, 26, 1937.
2. Lohmann, K.: Chemische Vorgänge bei der Muskelkontraktion. Zeit. angew. Chem. *50*, 221, 1937.
3. Lohmann, K., and Schuster, Ph.: Untersuchungen über die Cocarboxylase. Biochem. Zeit. *294*, 188, 1937.
4. Lohmann, K.: Beziehungen zwischen Fermenten und Vitaminen. Acta. Brev. Neerl. *7*, 108, 1937.
5. Peters, R. A.: Cocarboxylase and Vitamin B_1 in Brain. Chem. and Ind. *56*, 934, 1937.
6. Lipschitz, M. A., Potter, V. R., and Elvehjem, C. A.: Relation of Vitamin B_1 to Cocarboxylase. Biochem. J. *32*, 474, 1938.
7. Stern, K. G., and Hofer, J. W.: Synthesis of Cocarboxylase from Vitamin B_1. Enzymologia *3*, 82, 1937.
8. Tauber, H.: Enzymic Synthesis of Cocarboxylase. Science *86*, 180, 1937.

9. von Euler, H., and Vestin, R.: Enzymatische Synthese von Cocarboxylase aus Vitamin B_1 und Phosphat. Naturwissensch. *25*, 416, 1937.
10. Kuhn, R., Rudy, H., and Wagner-Jauregg: Über Lactoflavin (Vitamin B_2). Ber. deut. chem. Ges. *66*, 1950, 1933.
11. Stern, K. G., and Holiday, E. R.: Die Photo-flavine, eine Gruppe von Alloxazin-Derivaten. Ber. deut. chem. Ges. *67*, 1442, 1934.
12. Karrer, P., Salmon, H., Schopp, K., Schlitter, E., and Fritzsche, H.: Ein neues Bestrahlungsprodukt des Laktoflavine: Lumichrom. Helv. chim. Acta. *17*, 1010, 1934.
13. Kuhn, R., Wagner-Jauregg, Th., van Klaveren, F. W., and Vetter, H.: Über einen gelben, schwefelhaltigen Farbstoff aus Hefe. Zeit. physiol. Chem. *234*, 196, 1935.
14. Barger, G., Bergel, F., and Todd, A. R.: A Crystalline, Fluorescent Dehydrogenation Product from Vitamin B_1. Nature *136*, 259, 1935.
15. Barger, G., Bergel, F., and Todd, A. R.: Über das Thiochrom aus Vitamin B_1 (Aneurin). Ber. deut. chem. Ges. *68*, 2257, 1935.
16. Kuhn, R., and Vetter, H.: Zur Kenntnis des Thiochrom. Ber. deut. chem. Ges. *68*, 2375, 1935.
17. Makino, K., and Imai, T.: Bemerkungen über die Chemie des antineuritischen Vitamins. Zeit. physiol. Chem. 239, I, 1936.
18. Williams, R. R.: Structure of Vitamin B_1. Jour. Amer. Chem. Soc. *58*, 1063, 1936.
19. Bergel, F., and Todd, A. R.: The Structure of Aneurin and Thiochrome. Nature *138*, 76, 1936.
20. Todd, A. R., Bergel, F., Fraenkel-Conrat, H. L., and Jacob, A.: Aneurin. Part VI. A Synthesis of Thiochrome and Related Compounds. Jour. Chem. Soc., p. 1601, 1936.
21. Buchman, E. R., and Williams, R. R.: Studies of Crystalline Vitamin B_1. IX. The Action of Concentrated Hydrochloric Acid. Jour. Amer. Chem. Soc. *57*, 1751, 1935.
22. Bergel, F., and Todd, A. R.: Aneurin. Part VIII. Some Analogues of Aneurin. Jour. Chem. Soc. p. 1504, 1937.
23. Knight, B. C. J. G.: The Nutrition of *Staphylococcus Aureus*. The Activities of Nicotinamide, Aneurin (Vitamin B_1) and Related Compounds. Biochem. J. *31*, 966, 1937.
24. Andersag, H., and Westphal, K.: Über die Synthese des antineuritischen Vitamins. Ber. deut. chem. Ges. *70*, 2035, 1937.
25. British patent 471,416 to I. G. Farbenindustrie Aktiengesellschaft. Accepted Aug. 30, 1937. French Patent 816,432 to I. G. Farbenindustrie Aktiengesellschaft; published August 7, 1937.
26. Todd, A. R., Bergel, F., and Karimullah: Aneurin. Part IV. 5-Thioformamido Pyrimidines. Jour. Chem. Soc. p. 1557, 1936.
27. Todd, A. R., and Bergel, F.: Aneurin. Part V. The Synthesis of 3-Pyrimidylthiazolium Salts, Including an Isomer of Aneurin. Jour. Chem. Soc. p. 1559, 1936.

CHAPTER XV

PYRIMIDINES ASSOCIATED WITH THIAMIN

Chemical differences between the natural substances necessary for plants and those needed by animals were noted by Schopfer (1), and it was presently discovered that the requirements of the two kingdoms are in general strikingly different in one particular. Animals can utilize only the fully synthesized vitamin; plants can often utilize a mixture of the intermediates, which have been used in the artificial synthesis, fully as well as the final chemical combination of them. This observation was made independently by Robbins and Kavanagh (2) and by Schopfer and Jung (3).

This statement needs some qualification, for it has been found that very large doses by mouth of a mixture of the intermediates is effective in relieving polyneuritis in pigeons. Apparently from the large quantities necessary there is possibility of an inefficient synthesis in the alimentary tract, perhaps by microorganisms, or elsewhere in the body (4). On the other hand, some ten species of Phytophthora are unable to use the thiamin intermediates, thus resembling animals in their requirements (5).

In general, however, a mixture of the intermediates is effective for plants. In a few cases the pyrimidine alone is effective; in a somewhat larger number the thiazole alone is effective. In such instances it may be that the inoculum already contains a sufficiency of one intermediate or the other. However, when both intermediates are found to be required, the optimum effects are produced if the two intermediates are supplied in equimolecular proportions. This strongly suggests that plants can use the intermediates to synthesize thiamin, and that it is the latter substance, or a derivative of it such as the pyrophosphate, which constitutes the physiologically significant entity.

That such synthesis of thiamin actually takes place has been rendered very probable in one instance by the fact that a synthetic medium in which Torula had been grown has been thereby rendered capable of supporting the growth of Phycomyces in which the latter was not originally able to grow. One is tempted

to think that nature follows the same course in the final step of synthesis as that which has been found most advantageous in artificial manufacture, but a conclusion on this point is scarcely warranted at present.

The efficacy of the intermediates soon provoked a question as to what variants of the intermediates might be permissible. An early study of this sort was that of Knight (6), who found that thiamin plus nicotinic acid were essential for the growth of Staphylococcus aureus. In admixture with the proper thiazole, 2-methyl-6-amino-5-aminomethylpyrimidine and the corresponding 5-thioformamidopyrimidine were found active in the same sense as the vitamin. The compounds tested, which proved ineffective in admixture with the thiazole, were 2-methyl-6-oxy-5-hydroxymethylpyrimidine, 2-methyl-6-oxy-5-aminomethylpyrimidine and 2-oxy-6-aminopyrimidine (cytosine). Evidently only those pyrimidines which constitute fragments of the vitamin or can be readily hydrolyzed to such fragments are physiologically potent. Similar results were reported by Sinclair for the fungus Phycomyces (7). The 5-aminomethylpyrimidine and its thioformamido derivative corresponding to the vitamin were active, but the 6-oxy counterpart of the second was not.

Further papers along this line are constantly appearing. Those pyrimidines which have been found effective either with or without admixture of the proper thiazole component all contain an amino group in position 6 and a methyl group in position 2.* In position 5 aminomethyl, chloromethyl, bromomethyl, ethoxymethyl and hydroxymethyl appear nearly equally efficient. The thioformamido in position is qualitatively adequate but not as favorable quantitatively in most cases. Perhaps this group has some toxic action. It is more probable that it partially undergoes condensation with the adjacent amino group and thus becomes unavailable for hydrolysis. It has been noted that solutions of the compound are more effective if they are sterilized otherwise than by heat. The most extensive paper in the field is that of Robbins and Kavanagh, who dealt with Phycomyces (8). They have reported on the following pyrimidines:

1. 2-methyl-5-bromomethyl-6-amino	positive
2. 2-methyl-5-ethoxymethyl-6-amino	positive
3. 2-methyl-5-aminomethyl-6-amino	positive
4. 2-methyl-5-ethoxymethyl-6-oxy	negative
5. 2-methyl-6-amino	negative
6. 2,6-dichloro-5-chloromethyl-4-methyl	negative
7. 2,6-dioxy-5-hydroxy methyl-4-methyl	negative
8. 2-methyl-5-thioformamidomethyl-6-amino	50% positive

* Very recently W. J. Robbins (private communication) has found an ethyl group in position 2 is effective in some instances.

9.	2,4-dimethyl-6-amino	negative
10.	2,4,6-trimethyl	negative
11.	2-methylmercapto-4-methyl-6-oxy	negative
12.	2-phenyl-4-methyl-6-carboxylic acid	negative
13.	2-phenyl-4,6-dicarboxylic acid	negative
14.	4-methyl-5-hydroxymethyluracil	negative
15.	2-amino-5-methyl-1,3,4-thiodiazine	negative
16.	2-thioorotic acid	negative
17.	2-thio-4-methyluracil	negative
18.	orotic acid	negative
19.	5-iodouracil	negative
20.	uracil-4-aldehyde	negative
21.	2-thiouracil	negative
22.	uracil-5-carboxylic acid	negative
23.	1,4-dimethyluracil	negative
24.	2-thioimino barbituric acid	negative
25.	5-ethyl-4-methyluracil	negative
26.	5-bromouracil	negative
27.	2-thio-4-hydroxymethyluracil	negative
28.	4,5-diomethyluracil	negative
29.	2-methyl-6-mercapto	negative *
30.	2,5-dimethyl-6-amino	negative *
31.	2,5-dimethyl-6-oxy	negative *
32.	2-methyl-4-amino-6-hydroxy	negative *
33.	uracil	negative *

* Quoted from Schopfer (9).

Several instances have been cited in which analogues of thiamin containing the correct thiazole group in chemical combination with a wrongly constituted second nucleus have nevertheless been effective in admixture with one of the correct pyrimidines, *e.g.*, No. 2 above. Apparently, in some instances, the organism can split apart the two nuclei and use the properly constituted portion to combine with another suitably constituted intermediate to make thiamin. The tentatively known examples of such partially reconvertible condensates are 3-(2, 6-dichloro-4-methyl-pyrimidine-5-methyl)-4-methyl-5-β-hydroxyethylthiazolium chloride (Bowman), 3-benzyl-4-methyl-5-β-hydroxyethylthiazolium chloride, and 3-(4′, 5′-methylimidazole)-4-methyl-5-β-hydroxyethylthiazolium hydrochloride (9). Synthesis of thiamin from the appropriate pyrimidine and thiazole by the agency of plants is relatively well established (see Chapter XXVIII).

The synthetic chemistry of the pyrimidines which are of interest in connection with thiamin has been touched upon on page 150. The general method most suitable for the purpose is the condensation, by means of sodium hydroxide or sodium ethyl-

ate, of an appropriate β keto ester with an amidine, *e.g.*, acetamidine if a methyl group is desired in position 2 of the resulting pyrimidine. Urea, thiourea, or ethyl pseudo-thiourea are most often used if substituents other than alkyls or aryls are desired in this position. The 6-oxypyrimidine resulting from such condensation is converted with $POCl_3$ to the corresponding chlor derivative, and this in turn to the amino derivative by means of alcoholic ammonia. In certain instances it has been feasible to substitute a β keto nitrile for the original β keto ester and thus obtain the aminopyrimidine directly. This method is of less general applicability, especially if there are somewhat unstable groups on the carbon atom which lies between the keto and nitrile groups.

The isolation of the 2-alkyl-6-oxypyrimidines is often difficult. One sometimes makes use of the principle that oxypyrimidines are amphoteric and are held in aqueous solution by alkali or by acid. One can therefore extract with an immiscible solvent first in alkaline solution to remove bases, then in acid solutions to remove acidic impurities, and finally in neutral solution to extract the oxypyrimidine from the aqueous phase. Repeated extractions are usually necessary, as these pyrimidines are rarely as freely soluble in immiscible solvents as they are in water. Sublimation *in vacuo* is invaluable for purifications. Once pure, the substances crystallize well. The chlorpyrimidines are often volatile enough to require precautions against loss when removing excess $POCl_3$. The aminopyrimidines of this class may be extracted from alkaline solution by persistent use of immiscible solvents.

The 6-oxypyrimidines having oxy-, thio-, or ethylthio substituents are easily isolated as a rule by rendering the aqueous reaction mixture faintly acid, as they are sparingly soluble under these conditions and readily separate in a crystalline condition.

For the convenience of the reader we summarize below descriptions of new pyrimidines (and a few old ones) which have been prepared in this connection.

Some Pyrimidines Associated with the Study of Thiamin

1. $C_5H_6N_2O$ 2-methyl-6-hydroxypyrimidine. From formyl acetic ester and acetamidine (25).
2. $C_5H_6N_2O$ 2-methyl-6-hydroxypyrimidine, m. 148–9°. From 4-methyl-2-thiouracil and H_2O_2 (13).
3. $C_5H_6N_2O$ 5-methyl-6-hydroxypyrimidine, m. 153–4°; sublimes at 100° *in vacuo*. From thiothymine and H_2O_2 (13).
4. $C_5H_7N_2O_2$ Thyminylamine from thyminylurethan or isocyanate (28). M. 265°.
5. $C_5H_7N_3$ 4-methyl-6-aminopyrimidine, m. 194–5°. From 4-methyl-6-chloro-pyrimidine (13).

6. $C_5H_7N_3$ 2-methyl-6-aminopyrimidine from 2-methyl-6-chloropyrimidine (25).
7. $C_5H_7N_3$ 5-methyl-6-aminopyrimidine, m. 175–6°; sublimes at 110° *in vacuo*. From 5-methyl-6-chloropyrimidine (13).
8. $C_6H_4ClN_3$ 4-chloro-5-cyano-2-methylpyrimidine, m. 63–4°. From 4-hydroxy-5-hydroxy-5-cyano-2-methylpyrimidine and $POCl_3$ (20).
9. $C_6H_5N_3O$ 4-hydroxy-5-cyano-2-methylpyrimidine, m. 233–5°. From ethyl-α-cyano-β-acetamidinoacrylate and aq. NaOH (20).
10. $C_6H_5Cl_3N_2$ 2,4-dichloro-5-chloromethyl-6-methylpyrimidine, m. 38–9°. From 2,6-dihydroxy-5-hydroxymethyl-6-methylpyrimidine and $POCl_3$ (19). Used for preparing thiochrome derivatives.
11. $C_6H_5Cl_3N_2$ 2,4-dichloro-5-chloromethyl-6-methylpyrimidine (XXXVI), m. 39°. From 2,4-dihydroxy-5-hydroxymethyl-6-methylpyrimidine* and PCl_5 in $POCl_3$ (10).
12. $C_6H_5Cl_2IN_2$ 2,4-dichloro-5-iodomethyl-6-methylpyrimidine, m. 93.5–4.5°. From 2,4-dichloro-5-chloromethyl-6-methylpyrimidine in acetone and NaI in acetone (18); m. 90° (10).
13. $C_6H_5Cl_2N_3O_2$ 2,4-dichloro-5-nitro-4-amino-6-ethyl pyrimidine from 5-nitro-6-methyluracil + $POCl_3$ (26); m. 31°.
14. $C_6H_6N_4$ 4-amino-5-cyano-2-methylpyrimidine, m. 249°. From 4-chloro-5-cyano-2-methylpyrimidine. Also from 4-amino-2-methylpyrimidyl-(5)-carboxyamide and $POCl_3$ (20). From acetamidine and ethoxymethylenemalonic acid dinitrile, m. 249°; picrate, m. 208° (decompn.) (23, 24).
15. $C_6H_6Cl_2N_2$ 4-chloro-5-chloromethyl-2-methylpyrimidine, m. 54°. From 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine and $POCl_3$ (19).
16. $C_6H_7N_3O_4$ 5-nitro-6-ethyluracil from 6-ethyluracil with fuming HNO_3 and H_2SO_4 (26); m. 230°.
17. $C_6H_7ClN_2$ 2,5-dimethyl-6-chloropyrimidine. Very volatile. From 2,5-dimethyl-6-hydroxypyrimidine and $POCl_3$ (13).
18. $C_6H_7ClN_2$ 4,5-dimethyl-6-chloropyrimidine, m. 52°; sublimes at 50° *in vacuo*. From 4,5-dimethyl-6-hydroxypyrimidine and $POCl_3$ (13).
19. $C_6H_7Cl\ N_4O_2$ 2-chloro-5-nitro-4-amino-6-ethylpyrimidine from No. 13 above with cold NH_4OH (26); m. 140°–141°.
20. $C_6H_7N_3O_2S$ 5-thioformamido-4-methyluracil; hydrate m. 260–2°. From 5-amino-4-methyluracil and dithioformic acid in dioxane under reflux (17).
21. $C_6H_8N_2O$ 4,5-dimethyl-6-hydroxypyrimidine, m. 202–3°. From From 2-thio-4,5-dimethyl-6-hydroxypyrimidine and H_2O_2 (13).
22. $C_6H_8N_2O$ 2,5-dimethyl-6-hydroxypyrimidine, m. 174°; sublimes at 125° *in vacuo*. From ethyl sodioformylpropionate and acetamidine hydrochloride (13).
23. $C_6H_8N_2O_2$ 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine, m. 215–6°. From 2-hydroxy-5-aminomethyl-2-methylpyrimidine and $NaNO_2$ (10).

* Kircher, *Ann. 385*, 293, 1911.

24. $C_6H_8N_2O_2$ 6-ethyluracil from 2-thio-6-ethyluracil + dil. HNO_3, (26) m. 205°.
25. $C_6H_8N_4O$ 4-amino-2-methylpyrimidine-5-carboxyamide, m. 264–5°. From ethyl-4-amino-2-methylpyrimidine-5-carboxylate shaken with aq. NH_3 at room temp. for 36 hours (20).
26. $C_6H_8ClN_3$ 6-chloro-2-amino-4-ethylpyrimidine, m. 120–1°. From 2-amino-6-hydroxy-4-ethylpyrimidine and $POCl_3$ (17).
27. $C_6H_8BrN_3$ 2-methyl-6-amino-5-bromomethylpyrimidine. From 2-methyl-6-amino-5-ethoxymethylpyrimidine and HBr in HAc; hydrobromide, m. 192–3° (15). From 2-methyl-6-amino-5-hydroxymethylpyrimidine and HBr; hydrobromide (XXVII), m. 213° (decompn.) (10).
28. $C_6H_8ClN_3$ 2,6-dimethyl-4-chloro-5-aminopyrimidine, m. 80°; picrate, m. 169°. From 2,6-dimethyl-4-hydroxy-5-aminopyrimidine and PCl_5 in $POCl_3$ (10).
29. $C_6H_8BrN_3$ 4-amino-5-bromomethyl-6-methylpyrimidine. From 4-amino-5-hydroxymethyl-6-methylpyrimidine and HBr; hydrobromide (XXIX), m. 210–2° (decompn.) (10).
30. $C_6H_8N_4S$ 6-amino-5-thioformamido-4-methylpyrimidine, m. 168° (evolution of H_2S), remelts at 230°. From 5,6-diamino-4-methylpyrimidine and HCS_2K (17).
31. $C_6H_8N_2OS$ 2-thio-4, 5-dimethyl-6-hydroxypyrimidine, m. above 255°. From thiourea and ethyl-α-methylacetonacetate by condensation with C_2H_5ONa (13).
32. $C_6H_8N_2OS$ 6-ethyl-2-thiouracil from ethyl propionyl acetate and thiourea (26), m. 228°.
33. $C_6H_8ClN_3O$ 2-chloro-4-amino-5-hydroxymethyl-6-methylpyrimidine, m. 179°. From 2,4-dichloro-5-acetoxymethyl-6-methylpyrimidine and NH_3. Separated from 2-amino-4-chloro-5-hydroxy-6-methylpyrimidine by treatment with Zn dust (10).
34. $C_6H_8N_2O_4S$ 2-methyl-6-hydroxypyrimidine-5-methyl sulfonic acid, m. above 360° (12). From 2-methyl-5-ethoxymethyl-6-hydroxypyrimidine, Na_2SO_3 and SO_2 (14).
35. $C_6H_9N_3$ 2,5-dimethyl-6-aminopyrimidine, m. 201–2°, sublimes at 80°; picrate, m. 222°. From 2,5-dimethyl-6-chloropyrimidine (13).
36. $C_6H_9N_3$ 2,5-dimethyl-6-aminopyrimidine, m. 202°; picrate, m. 221–3°. From 2-methyl-6-aminopyrimidine-5-methyl sulfonic acid (from vitamin) by reduction with Na in liquid NH_3 (14). From 2,5-dimethyl-6-chloropyrimidine with alcoholic NH_3 (13).
37. $C_6H_9N_3$ 4,5-dimethyl-6-aminopyrimidine, m. 229–31°. From 4,5-dimethyl-6-chloropyrimidine (13).
38. $C_6H_9N_3O$ 2,6-dimethyl-5-hydroxy-5-aminopyrimidine (XVII), m. 194°. From 2,6-dimethyl-4-hydroxy-5-phenylazopyrimidine and $NaHSO_3$ (10).
39. $C_6H_9N_3O$ 2-methyl-3-amino-5-hydroxymethylpyrimidine (XXVI), m. 194°, HCl salt, m. 224°. From 2-methyl-4-amino-5-aminomethylpyrimidine dihydrochloride and $NaNO_2$ (10).
40. $C_6H_9N_3O$ 4-hydroxy-5-aminomethyl-2-methylpyrimidine; HCl

salt, m. 278–82°; HBr salt, m. 270°. From 4-hydroxy-5-urethanomethyl-2-methylpyrimidine HCl salt and conc. HCl (19).

41. $C_6H_9N_3O$ 2-amino-6-hydroxy-4-ethylpyrimidine, m. 247–8°. From ethyl propionylacetate and guanidine carbonate (17).
42. $C_6H_9N_3O$ 4-amino-5-hydroxymethyl-6-methylpyrimidine (XL), m. 166°. From 6-methyl-4-amino-5-aminomethylpyrimidine 2 HCl salt and $NaNO_2$. Also from 2-chloro-4-amino-5-hydroxymethylmethylpyrimidine and Zn dust (10).
43. $C_6H_9N_3O$ 6-methyl-4-amino-5-hydroxymethylpyrimidine (XV), m. 166°. From 6-methyl-4-amino-5-aminomethylpyrimidine dihydrochloride and $NaNO_2$ (10).
44. $C_6H_9N_4Cl$ 2-chloro-4,5-diamino-6-ethylpyrimidine from No. 19 above with $SnCl_2$ in HCl (26); m. 203°.
45. $C_6H_9N_5S$ 2,6-diamino-5-thioformamido-4-methylpyrimidine, m. 235° (evolution of H_2S). From 2,5,6-triamino-4-methylpyrimidine and HCS_2K (17).
46. $C_6H_9N_3O_3S$ 2-methyl-4-aminopyrimidyl-5-methylsulfonic acid, m. 440°. From the vitamin treated with Na_2SO_3 and SO_2 at *p*H 5 (11). 2-methyl-4-amino-5-pyrimidylmethylsulfonic acid (XIV). From 2-methyl-4-amino-5-bromomethylpyrimidine, $NaHSO_3$, and SO_2 to saturation (23).
47. $C_6H_{10}N_4$ 2,6-diamino-4-ethylpyrimidine, m. 160–1°. From 6-chloro-2-amino-4-ethylpyrimidine and alc. NH_3 in sealed tube at 180° for 6 hr. (17).
48. $C_6H_{10}N_4$ 2,6-dimethyl-4,5-diaminopyrimidine hydrochloride (XI); hydrochloride + $\frac{1}{2}$ H_2O, m. 271°; free base, m. 248°; picrate, m. 235°. From 2,6-dimethyl-4-chloro-5-aminopyrimidine with CH_3OH and NH_3 under pressure, m. 238° (10).
49. $C_6H_{10}N_4$ 2-methyl-4-amino-5-aminomethylpyrimidine; picrate, m. 224.5°; dihydrochloride, m. 265°. By oxidation of vitamin HCl with $Ba(MnO_4)_2$ (22). From cleavage of vitamin with liq. NH_3 to free base, m. 211–5°; picrate, m. 225° (14).
50. $C_6H_{10}N_4$ synthetic duplication of 49; dihydrochloride XIX, m. 268–9°; picrate, m. 224–5°; sulfate, m. 276°; free base, m. 132°. From 2-methyl-4-aminopyrimidyl-(5)-acetamide and KOBr. Condenses with benzaldehyde (10). Formyl compound made by treating free base with ethyl formate under pressure to $C_7H_{10}N_4O$, m. 224°: By hydrogenation of 4-amino-5-cyano-2-methylpyrimidine with palladium; dihydrochloride, m. 262°; m. 264–5° (12); picrate, m. 224.5° (decompn.) (23, 24).
51. $C_6H_{10}N_4$ 6-methyl-4-amino-5-aminomethylpyrimidine; picrate, m. 238° (decompn.); hydrochloride, m. 277°. From 4-amino-5-bromomethyl-6-methylpyrimidine and NH_3 (10). Also from 6-methyl-4-aminopyrimidyl-(5)-acetamide and KOBr. Condenses with benzaldehyde.
52. $C_6H_{10}N_4$ 5-ethyl-4,6-diaminopyrimidine, m. 245°; picrate, m. 165–7°. From 4-iodo-5-ethyl-6-aminopyrimidine and alc. NH_3 at 220° for 15 hr. (14). Condensation product of benzil and above, m. 207° (10).

53. $C_7H_8N_2O_3$ 2-methyl-6-hydroxypyrimidine-5 acetic acid (28). From acetamidine and ethylformyl succinate, m. 245°–6°. Ethyl ester, m. 179°–180°. Amide, m. 242°.
54. $C_7H_8N_2O_3S$ 2-mercapto-6-methyl-4-hydroxypyrimidyl-(5)-acetic acid (XXXI), m. 296°. From thiourea, acetosuccinic ester, and C_2H_5ONa (10).
55. $C_7H_9N_3OS$ 4-hydroxy-5-thioformamidomethyl-2-methylpyrimidine, m. 199–200°. From 4-hydroxy-5-aminomethyl-2-methylpyrimidine (19).
56. $C_7H_9N_3O_2S$ 5-thioacetamido-4-methyluracil, m. 265–7°. From 5-amino-4-methyluracil and thioacetic acid (17).
57. $C_7H_{10}N_4S$ 4-amino-5-thioformamidomethyl-2-methylpyrimidine, m. 187° (decompn.). From 4-amino-5-aminomethyl-2-methylpyrimidine and HCS_2K (20). From the formyl derivative of 4-amino-5-aminomethyl-2-methylpyrimidine and P_2S_5, m. 193° (10).
58. $C_7H_{10}N_4S$ 6-amino-5-thioformamido-4-ethylpyrimidine, m. 178° (evolution of H_2S). By reduction of 2-chloro-5,6-diamino-4-ethylpyrimidine to 5,6-diamino-4-ethylpyrimidine and treatment with HCS_2K (17).
59. $C_7H_{10}N_4O$ 6-methyl-4-aminopyrimidyl-(5)-acetamide, m. 223°. From ethyl-6-methyl-4-chloropyrimidyl-(5)-acetate and NH_3 (10).
60. $C_7H_{10}N_4O$ 2-methyl-4-aminopyrimidyl-(5)-acetamide, m. 250° (XXIII). From ethyl-2-methyl-4-chloropyrimidyl-(5)-acetate and NH_3 (10). Readily hydrolyzed. Corresponding acid, m. 270°. $C_8H_{11}O_2N_3$ 2-methyl-4-methoxypyrimidyl-(5)-acetamide, m. 201° by side reaction.
61. $C_7H_{10}N_4O_2$ 4-hydroxy-2-methylpyrimidyl-(5)-acetohydrazide, m. 246°. From ethyl 4-hydroxy-2-methylpyrimidyl-(5)-acetate and hydrazine hydrate (19).
62. $C_7H_{11}N_3O$ 2-methyl-4-methoxy-5-aminomethylpyrimidine (XXV), b. 110–6°; 2 HCl salt, m. 150–1°; picrate, m. 188°. From 2-methyl-4-methoxypyrimidyl-(5)-acetamide and KOBr (10).
63. $C_7H_{11}N_5O_5$ 4-hydroxy-5-hydroxymethylpyrimidine nitrate (?), m. 146–53° (decompn.). From vitamin B_1 hydrochloride and HNO_3 (21).
64. $C_8H_8Cl_2N_2O_2$ 2,4-dichloro-5-acetoxymethyl-6-methylpyrimidine (XXXVII), m. 55°, b_4 141°. From 2,4-dichloro-5-acetoxy-methyl-6-methylpyrimidine and AgOAc (10).
65. $C_8H_{10}N_2O_3$ Ethyl 4-hydroxy-2-methylpyrimidyl-(5)-carboxylate, m. 191°. From acetamidine hydrochloride, ethyl ethoxymethylene malonate, and C_2H_5ONa (20).
66. $C_8H_{11}N_3O_2$ 4-hydroxy-5-acetamidomethyl-2-methylpyrimidine, m. 219–20°. From 4-hydroxy-5-aminomethyl-2-methylpyrimidine HCl, NaAc, and acetic anhydride (19).
67. $C_8H_{11}N_3O_2$ Ethyl 4-amino-2-methylpyrimidyl-(5)-carboxylate, m. 120°. From ethyl 4-chloro-2-methylpyrimidyl-(5)-carboxylate and alc. NH_3 (20). Chloro compd. prepared from the corresponding hydroxy compd. and $POCl_3$.

68. $C_8H_{11}ClN_2O$ 2-methyl-5-ethoxymethyl-6-chloropyrimidine. From 2-methyl-5-ethoxymethyl-6-hydroxypyrimidine, $b_{0.5}$ 72–3° (15).
69. $C_8H_{12}N_2O_2$ 2-methyl-5-ethoxymethyl-6-hydroxypyrimidine. From sodioformyl-ethoxy-propionate, acetamidine, and C_2H_5ONa (15), m. 175–6°.
70. $C_8H_{12}N_4S$ 4-amino-5-thiacetamido-2-methylpyrimidine, m. 228–9°. From 4-amino-5-aminomethyl-2-methylpyrimidine and dithioacetic acid (18).
71. $C_8H_{13}N_3O$ 2-methyl-5-bromomethyl-6-aminopyrimidine. From 2-methyl-5-ethoxymethyl-6-chloropyrimidine and alc. NH_3; m. 89.5–90.5° (15).
72. $C_9H_{12}N_2O_3$ Ethyl 2,6-dimethyl-4-hydroxypyrimidyl-(5)-carboxylate, m. 164°; free acid, m. 220° (decompn.); methyl ester, m. 160–2°. From ethyl 2,6-dimethyl-4-hydroxy-5,6-dihydropyrimidyl-(5)-carboxylate and bromine (23, 24).
73. $C_9H_{11}ClN_2O_2$ Ethyl 6-methyl-4-chloropyrimidyl-(5)-acetate (XXII), m. 40–1°. From 2-methyl-4-hydroxypyrimidyl-(5)-acetate and $POCl_3$ (10).
74. $C_9H_{11}ClN_2O_2$ Ethyl 6-methyl-4-chloropyrimidyl-(5)-acetate, $b_{1.5}$ 116–7°. From ethyl 6-methyl-4-hydroxypyrimidyl-(5)-acetate and $POCl_3$ (10).
75. $C_9H_{12}N_2O_3$ Ethyl 2-methyl-4-hydroxypyrimidyl-(5)-acetate, m. 179°. From formyl succinate and acetamidine hydrochloride in C_2H_5ONa (10).
76. $C_9H_{12}N_2O_3$ Ethyl 6-methyl-4-hydroxypyrimidyl-(5)-acetate, m. 153°, distg. 203–5° at 0.5 mm. From ethyl 2-mercapto-6-methyl-4-hydroxypyrimidyl-(5)-acetate, $Pb(AcO)_2$ and H_2O_2 (10).
77. $C_9H_{12}N_2SO_3$ Ethyl 2-mercapto-6-methyl-4-hydroxypyrimidyl-(15)-acetate (XXXII), m. 218°. From 2-mercapto-6-methyl-4-hydroxypyrimidyl-(5)-acetic acid, C_2H_5OH and H_2SO_4 (10).
78. $C_9H_{13}N_3O_2$ 2,6-dimethyl-4-amino-5-carbethoxypyrimidine, m. 129°. From ethyl 2,6-dimethyl-4-hydroxypyrimidyl-(5)-carboxylate and $POCl_3$ (23).
79. $C_9H_{13}N_3O_3$ 4-hydroxy-5-urethanomethyl-2-methylpyrimidine, m. 173°; HCl salt, m. 209°. From 4-hydroxy-2-methylpyrimidyl-(5)-acetohydrazide, amylnitrite and HCl (19).
80. $C_9H_{14}N_2O_3$ Ethyl 2,6-dimethyl-4-hydroxy-5,6-dihydropyrimidyl-(5)-carboxylate; HCl salt, m. 205° (decompn.); free acid, m. 294° (decompn.). From acetamidine, ethylidene malonic ester, and C_2H_5ONa (23).
81. $C_{12}H_{11}Cl_4N_5$ Bis [2,4-dichloro-6-methylpyrimidyl-(5)-]-methylamine, m. 162–3°. From 2,4-dichloro-5-chloromethyl-6-methylpyrimidine and alc. NH_3 (19).
82. $C_{12}H_{12}N_4O$ 2,6-dimethyl-4-hydroxy-5-phenylazopyrimidine (XVI), m. 186°. From phenylazoacetoacetic ester, acetamidine HCl and C_2H_5ONa (10).

BIBLIOGRAPHY

1. Schopfer, W. H.: Etude sur les Facteurs de Croissance. Action de la Vitamine Cristallisée B_1 et de l'Extrait de Germe de Blé sur Rhizopus et d'Autres Mucorinées. Zeit. f. Vitaminforsch *4*, 187, 1937.
2. Robbins, W. J., and Kavanagh, F.: Intermediates of Vitamin B_1 and Growth of *Phycomyces*. Proc. Nat. Acad. Sci. 23, 499, 1937.
3. Schopfer, W. H., and Jung, A.: Physiologie Vegetale. L'Action des Produits de Desintegration de l'Aneurine sur Phycomyces. Le Second Facteur de Croissance des Mucorinées. Compt. Rend. Acad. Sci. *204*, 1500, 1937.
4. Robbins, W. J., Bartley, M. A., Hogan, A. G., and Richardson, L. R.: Pyrimidine and Thiazole Intermediates as Substitutes for Vitamin B_1. Proc. Nat. Acad. Sci. *23*, 388, 1937.
5. Robbins, W. J.: Organisms Requiring Vitamin B_1. Proc. Nat. Acad. Sci. *24*, 54, 1938.
6. Knight, B. C. J. G.: The Nutrition of *Staphylococcus aureus*. The Activities of Nicotinamide, Aneurin (Vitamin B_1) and Related Compounds. Biochem. J. *31*, 966, 1937.
7. Sinclair, H. M.: Growth Factors for *Phycomyces*. Nature *140*, 361, 1937.
8. Robbins, W. J., and Kavanagh, F.: The Specificity of Pyrimidine for *Phycomyces Blakesleeanus*. Proc. Nat. Acad. Sci. *24*, 141, 1938.
9. Schopfer, W. H. La Spécificité d'Action de l'Aneurine sur *Phycomyces*. Le Rôle des Constituants de l'Aneurine et de leurs Produits de Substitution. Bull. Soc. bot. Suisse *47*, 460, 1937.
10. Andersag, H., and Westphal, K.: Über die Synthese des antineuritischen Vitamins. Ber. deut. chem. Ges. *70*, 2035, 1937.
11. Williams, R. R., Waterman, R. E., Keresztesy, J. C., and Buchman, E. R.: Studies of Crystalline Vitamin B_1. III. Cleavage of Vitamin with Sulfite. Jour. Amer. Chem. Soc. *57*, 536, 1935.
12. Williams, R. R., Buchman, E. R., and Ruehle, A. E.: Studies of Crystalline Vitamin B_1. VIII. Sulfite Cleavage. Chemistry of the Acidic Product. Jour. Amer. Chem. Soc. *57*, 1093, 1935.
13. Williams, R. R., Ruehle, A. E., and Finkelstein, J.: Studies of Crystalline Vitamin B_1. XV. C-methylated 6-amino- and 6-oxypyrimidines. Jour. Amer. Chem. Soc. *59*, 526, 1937.
14. Cline, J. K., Williams, R. R., Ruehle, A. E., and Waterman, R. E.: Studies of Crystalline Vitamin B_1. XVI. Identification of the Pyrimidine Portion. Jour. Amer. Chem. Soc. *59*, 530, 1937.
15. Cline, J. K., Williams, R. R., and Finkelstein, J.: Studies of Crystalline Vitamin B_1. XVII. Synthesis of Vitamin B_1. Jour. Amer. Chem. Soc. *59*, 1052, 1937.
16. Todd, A. R., Bergel, F., and Jacob, A.: Aneurin. Part III. Methyl α-chloro-γ-hydroxypropyl Ketone and Its Application to Thiazole Synthesis. Jour. Chem. Soc. p. 1555, 1936.

17. Todd, A. R., Bergel, F., and Karimullah: Aneurin. Part IV. 5-Thioformamido-pyrimidines. Jour. Chem. Soc. p. 1557, 1936.
18. Bergel, F., and Todd, A. R.: Aneurin. Part VIII. Some Analogues of Aneurin. Jour. Chem. Soc. p. 1504, 1937.
19. Todd, A. R., Bergel, F., Fraenkel-Conrat, H. L., and Jacob, A.: Aneurin. Part VI. A Synthesis of Thiochrome and Related Compounds. Jour. Chem. Soc. p. 1601, 1936.
20. Todd, A. R., and Bergel, F.: Aneurin. Part VII. A Synthesis of Aneurin. Jour. Chem. Soc. p. 364, 1937.
21. Windaus, A., Tschesche, R., and Grewe, R.: Über das antineuritische Vitamin. Z. physiol. Chem. *228*, 27, 1934.
22. Windaus, A., Tschesche, R., and Grewe, R.: Über das antineuritische Vitamin. Z. physiol. Chem. *237*, 98, 1935.
23. Grewe, R.: Über das antineuritische Vitamin. Z. physiol. Chem. *242*, 89, 1936.
24. Grewe, R.: Die Konstitution des Aneurins (Vitamin B_1). Naturwissenschaften *24*, 657, 1936.
25. Gabriel, S. Über 2-methyl pyrimidine. Ber. deut. chem. Ges. *37*, 3638, 1904.
26. Robinson, R., and Tomlinson, M. L.: Pyrimidines. Part II. Jour. Chem. Soc. p. 1283, 1935.
27. Cerecedo, L. R., and Pickel, F. D.: Pyrimidines I. 2-methyl-6-oxy-pyrimidine-5-acetic Acid and Its Derivatives. Jour. Amer. Chem. Soc. *59*, 1714, 1937.
28. Litzinger, A., and Johnson, T. B.: Pyrimidines. CLV. Synthesis of Thyminylamine and Its Conversion into Uracil. Jour. Amer. Chem. Soc. *58*, 1940, 1936.

CHAPTER XVI

THIAZOLES RELATED TO THIAMIN

Mention has already been made in the previous chapter of the fact that for many plant organisms a mixture of the intermediates is as effective as the chemical combination of them. In some instances the thiazole portion alone is effective. This phenomenon, which is of great interest from a biological standpoint, is discussed in Chapter XXVIII. What is perhaps of greater immediate interest to the chemist is the variations of the thiazole structure which certain organisms are able to utilize.

This phase of the subject began to develop with amazing rapidity soon after the first synthesis of thiamin was announced. It was a very natural outgrowth of the discovery that chemical combination of the two nuclei is often unnecessary. This fact permitted the study of the variants of each intermediate separately and allowed the chemist to escape, or at least postpone, the additional labor of effecting the final condensation and isolation of the pure thiamin analogues for biological tests. The final condensates will of course be necessary for trials upon animal organisms. Within a few short months the botanists have accordingly overshadowed the progress of the animal physiologists in determining the specificity of the thiamin molecule in natural processes.

Staphylococcus was the earliest plant organism to supply data as to the specificity of the tertiary thiazole structure (1). 4-methylthiazole was unable to act as a substitute for the thiazole of thiamin when used in conjunction with the proper pyrimidine. However Phycomyces presently proved unresponsive to a considerable list of substitute thiazoles, each being in use in conjunction with a properly constituted pyrimidine (2, 3). Other thiazoles were moderately active. The two groups are listed separately.

Thiazoles Inactive for Phycomyces

4-methylthiazole
4-methylthiazole acetic acid
4-methylthiazole-5-acetamide
4-methyl-5-β-carbethoxyethylthiazole

thiazolidine-4-carboxylic acid
2,4-dimethyl-5-β-hydroxyethylthiazole
4-methyl-5-β-hydroxypropyl-thiazole
2-keto-4-methyl-2:3-dihydrothiazole-2-benzylidine hydrazone
4,5-dimethylthiazole

Substitute Thiazoles Active for Phycomyces

4-methyl-5-β-ethoxyethylthiazole
4-methyl-5-γ-hydroxypropylthiazole *
4-methyl-5-β-chloroethylthiazole
2,4-dimethylthiazolepheniodide *

Of the four thiazoles exhibiting some activity two are of such a character as to be rather readily hydrolyzed to yield the thiazole of thiamin. These two showed pronounced activity though not equal to that of the substance of natural origin. The other two had less than 2 per cent of that activity, which may possibly be due to impurities present in them. The results are in general accord with those of Bonner (4), who tested also with Phycomyces the list of thiazoles which is referred to below. All of them except No. 10 gave negative results in admixture with the proper pyrimidine. The exception is one which could presumably hydrolyze readily to form the proper thiazole of the vitamin.

Thiazoles Tested by Bonner

1.	4-methylthiazole	
2.	4-methyl-5-ethylthiazole	
3.	4-methyl-thiazole-5-aldehyde	
4.	4-methyl-5-aceto-thiazole	
5.	4-methylthiazole-5-acetic acid	
6.	4-methyl-5-β-hydroxyethylthiazole ethiodide	(?)
7.	4-methyl-5-vinylthiazole	(100)
8.	4-hydroxymethyl-5-ethylthiazole	(100)
9.	4-methyl-5-bromethylthiazole	(100)
10.	4 methyl-5-β-chloroethylthiazole	(75)
11.	4 methyl-5-γ-hydroxypropylthiazole	(75)
12.	4 methyl-5-β-hydroxypropylthiazole	(75)
13.	4 methyl-5-α-hydroxyethylthiazole	(50)
14.	2,4-dimethyl-5-β-hydroxyethylthiazole	(30)

Bonner has also tested all of the above list of thiazoles as nutrilites for the growth of pea roots (4). The results are in quite marked contrast to those with Phycomyces. For pea roots Nos. 7 to 14, inclusive, are active to an extent which is indicated

* Only slightly active.

by the numbers in parentheses following each name. In each case the number indicates the approximate percentage of activity as compared with that of the natural thiazole. Bonner's conclusion is that for pea roots a hydroxyl group (or a group readily converted to hydroxyl) in a side chain is essential but that it need not occupy a specific position on the side chain. Nor is it necessary that the side chain containing the hydroxyl occupy a specific position.

It will be recalled that the hydroxyl is also essential for all other organisms so far as known but that the position of the hydroxyl is quite important for Phycomyces and, so far as tested, for animals. One is tempted to suppose from various other results such as those of Robbins (5) that organisms may make use of substitute thiazoles by converting them into the proper thiazole. However, Bonner's finding that for pea roots the presence, but not the precise position, of the hydroxyl is necessary strongly suggests that the substitute is being used as such. It will be of great interest to see whether or not secondary abnormalities of development occur at some stage of the life cycle when the organism is restricted to an unnatural thiazole.

The sole exception to the evidence for necessity of a side chain hydroxyl is vinyl thiazole. It does not seem improbable, however, that the vinyl group may be converted in the aqueous nutrient medium by addition of water across the double bond into an alcoholic group.

The principal contributors of thiazoles for the above botanical studies were Drs. E. R. Buchman, A. R. Todd, L. R. Cerecedo, and H. T. Clarke, to each of whom acknowledgments are made by the authors of the original papers.

The best synthesis of the natural thiazole is that of Buchman (6), consisting of the condensation of halogenated acetopropyl alcohol and thioformamide. Similar methods apply for most of the other thiazoles which are of interest in this connection. Variants of the Buchman method have employed halogenated acetopropyl esters, ethers, or halides in lieu of the corresponding alcohol. Thiourea has been substituted for thioformamide in effecting the condensation. In this case the 2-amino thiazole is obtained, which can be converted with nitrous acid to the 2-hydroxy; this hydroxyl group in turn is replaced by halogen by means of $POCl_3$ and the halogen by hydrogen by the use of a reduction process. The later steps of this synthesis are also common to another process which involves the condensation of the halogenated acetopropyl compound with a metallic thiocyanate in place of thioformamide. A 2-hydroxy thiazole is thus obtained. It is then necessary to replace the hydroxyl with hydrogen as above indicated. These methods are all roundabout and can scarcely be economical. They appear to

have been devised for purely scientific purposes or in some cases to escape infringement of patents. Representatives of each of the methods are found in the following tabulation of chemical data. The synthetic methods used in the preparation of many of the thiazoles mentioned in the above account of various tests of bioactivity have not yet been published, and the chemical data regarding them are not available.

Thiazoles Associated with the Study of Thiamin

1. C_4H_5NS 4-methylthiazole, b. 130°; ethiodide, m. 144.5°. From thioformamide and chloracetone (8).
2. $C_5H_5NO_2S$ 4-methylthiazole-5-carboxylic acid, sublimes at 255°; ethyl ester, m. 28°; methyl ester, m. 75°. From thioformamide and ethyl α-chloracetoacetate and saponification (8). Methyl ester, m. 73–4°, from the vitamin with HNO_3 (14).
3. C_5H_7NS 2,4-dimethylthiazole; ethiodide, m. 212° (decompn.). From thioacetamide and chloracetone (8).
4. C_6H_8ClNS 4-methyl-5-(β-chlorethyl)thiazole, b_3 74–5°; picrate, m. 139–40°; methiodide, m. 165°; chloroplatinate, m. 215–8° (decompn.). From 4-methyl-5-(β-hydroxyethyl)thiazole and HCl (6).
5. C_6H_8ClNS 4-methyl-5-(β-chlorethyl)thiazole; HCl salt, m. 127–8°; picrate, m. 138°. From 4-methyl-5-(β-ethoxyethyl)thiazole and HCl (8).
6. C_6H_9NOS 4-methyl-5-(β-hydroxyethyl)thiazole; picrate, m. 162–3°; picrolonate, m. 184° (decompn.); chloroplatinate, m. 181° (decompn.). From the vitamin treated with Na_2SO_3 at *p*H 5 (10). From chloracetopropyl alcohol and thioformamide in alcohol. Also from γ-bromo-γ-acetopropyl alcohol and thioformamide (6, 11).
7. C_6H_9NOS 4-methyl-5-(β-hydroxyethyl)thiazole, b_7 135°, b_3 123–4°; picrate, m. 163–4°. From 4-methyl-5-acetomethylthiazole by saponification with NaOH (9). From methyl-α-chloro-γ-acetoxy ketone and thioformamide; picrate, m. 162-3° (12). From 4-methyl-5-(β-chlorethyl)thiazole and H_2O (8).
8. $C_6H_{11}N_2OS$ 2-amino-4-methyl-5-(β-hydroxyethyl)thiazole, b_2 172–5°, m. 85–90°; picrate, m. 213°. From thiourea and methyl-α-chloro-γ-hydroxypropyl ketone (13).
9. $C_7H_9ClN_2S$ 4′-methylthiazole-3′,2′,1,2-(5-methyl-imidazol) hydrochloride, m. 242°. From 2-mercapto-4-methyl-imidazol and chloracetone by condensation by C_2H_5ONa (9).
10. $C_8H_{10}ClNO_2S$ 2-chloro-4-methyl-5-acetoxyethylthiazole, $b_{0.2}$ 103–5°. From 2-hydroxy-4-methyl-5-acetoxyethylthiazole and $POCl_3$ (9).
11. $C_8H_{11}NO_2S$ 4-methyl-5-acetoxyethylthiazole, b_5 112°; picrate, m. 133°. From 2-chloro-4-methyl-5-acetoxyethyl-thiazole by reduction with Zn dust and AcOH (9).
12. $C_8H_{11}NO_2S$ Ethyl 4-methylthiazole-5-acetate, b_3 107°–112°. Hydrobromide, m. 168°. From thioformamide + ethyl β bromo-

levulinate. Yields free acid by hydrolysis, m. 189°. Amide, m. 136°. Acethydrazide, m. 111° (7).

13. $C_8H_{11}NO_3S$ 2-hydroxy-4-methyl-5-acetoxyethylthiazole, m. 89°. From γ-aceto propylacetate by bromination and treatment with $Ba(CNS)_2$ (9).
14. $C_8H_{13}NOS$ 4-methyl-5-(β-ethoxyethyl)thiazole, b. 235° (decompn.); H_2PtCl_6 salt, m. 154–5°; $PtCl_4$ salt, m. 144–5°; picrate, m. 112°. From thioformamide and methyl-α-chloro-γ-ethoxypropyl ketone (8).
15. $C_{10}H_8N_2S$ 4′-methylthiazole-3′,2′,1,2-benzimidazol, m. 165°. From mercaptobenzimidazol and chloracetone condensed by C_2H_5ONa (9).
16. $C_{10}H_{10}INS$ 4-methyl-3-phenylthiazolium iodide, m. 241°. From thioformamide and chloracetone and further treatment with KI in H_2O (8).
17. $C_{11}H_{12}INS$ 2,4-dimethyl-3-phenylthiazolium iodide, m. 210°. From thioacetanilide and chloracetone and refluxing for 1.5 hr. with KI in H_2O (8).
18. $C_{13}H_{15}NOS$ 2,4-dimethyl-5-(β-phenoxyethyl)thiazole; picrate, m. 122°. From methyl-α-chloro-γ-phenoxypropyl ketone and thioacetamide warmed over free flame (12).

BIBLIOGRAPHY

1. Knight, B. C. J. G.: The Nutrition of *Staphylococcus aureus*. The Activities of Nicotinamide, Aneurin (Vitamin B_1) and Related Compounds. Biochem. J. 31, 966, 1937.
2. Robbins, W. J., and Kavanagh, F.: The Specificity of Thiazole for *Phycomyces Blakesleeanus*. Proc. Nat. Acad. Sci. *24*, 145, 1938.
3. Schopfer, W. H.: La Spécificité d'Action de l'Aneurine sur *Phycomyces*. Le Rôle des Constituants de l'Aneurine et de leurs Produits de Substitution. Bull. Soc. bot. Suisse *47*, 460, 1937.
4. Bonner, J.: Private communication.
5. Robbins, W. J.: Intermediates of Vitamin B_1 and the Growth of Torula. Plant Physiol. (In press.)
6. Buchman, E. R.: Studies of Crystalline Vitamin B_1. XIV. Sulfite Cleavage. The Thiazole Half. Jour. Amer. Chem. Soc. *58*, 1803, 1936.
7. Cerecedo, L. R., and Tolpin, J. G.: Thiazoles: (I) 4-methylthiazoleacetic Acid and Its Derivatives. Jour. Amer. Chem. Soc. 59, 1660, 1937.
8. Clarke, H. T., and Gurin, S.: Studies of Crystalline Vitamin B_1; XII. The Sulfur Containing Moiety. Jour. Amer. Chem. Soc. *57*, 1876, 1935.

9. Andersag, H., and Westphal, K.: Über die Synthese des antineuritischen Vitamins. Ber. deut. chem. Ges. *70*, 2035, 1937.
10. Buchman, E. R., Williams, R. R., and Keresztesy, J. C.: Studies of Crystalline Vitamin B_1. Sulfite Cleavage. Chemistry of the Basic Cleavage Product. Jour. Amer. Chem. Soc. *57*, 1849, 1935.
11. Grewe, R.: Die Konstitution des Aneurins (Vitamin B_1). Naturwissenschaften *42*, 675, 1936
12. Todd, A. R., Bergel, F., and Jacob, A.: Aneurin Part III. Methyl α-chloro-γ-hydroxypropyl Ketone and Its Application to Thiazole Synthesis. Jour. Chem. Soc. p. 1555, 1936.
13. Todd, A. R., Bergel, F., Fraenkel-Conrat, and Jacob, A.: Aneurin Part IV. A Synthesis of Thiochrome and Related Compounds. Jour. Chem. Soc. p. 1601, 1936.
14. Windaus, A., Tschesche, R., and Grewe, R.: Über das antineuritische Vitamin. Z. physiol. Chem. *228*, 27, 1934.

CHAPTER XVII

METHODS OF ASSAY OF THIAMIN

With such a confusing history as that outlined in Chapter X freshly in mind, the reader, even if inexperienced in this special field of nutritional studies, will recognize that there are extreme difficulties associated with the biological assay of any foodstuff. B_1, the earliest known member of the B group, can still be determined only with an approximate degree of precision. Many different methods of assay have been proposed and are still in use. The earliest involved the use of the domestic fowl and was patterned after Eijkman's original experiments. Schaumann and Funk substituted the pigeon and introduced the practice of observing the weight of the birds from day to day while they were being maintained upon an experimental diet. Soon after this the method based on the growth of young rats came into being.

The process of evolution has gone on in this fashion until there are today about twenty methods in more or less current use or actively advocated by certain groups of workers. Some of the methods are passing into obsolescence, but in numerous instances results obtained by one of the less suitable methods afford our only knowledge on the occurrence of the substance in particular foods. An evaluation of all the methods is therefore necessary to a full utilization of existing literature data.

The methods which deserve special discussion fall into the following classification:

I. Methods based on prevention of polyneuritis
   (*a*) Fowls on a polished rice diet. (Eijkmann and many other early workers)
   (*b*) Pigeons on polished rice or synthetic diets. (Funk and many others)
   (*c*) Rice birds on polished rice diet. (Jansen and Donath)
   (*d*) Chicks. (Elvehjem)
   (*e*) Rats. (Scheunert and Schieblich)

II. Methods based on rate of growth
   (*a*) Rat growth. (Sherman, Chick, Roscoe)
   (*b*) Mouse growth. (Cerecedo)

III. Methods based on weight maintenance of adult animals
   (*a*) Pigeons. (Seidell, Cowgill)

IV. Methods based on restoration of weight and appetite
  (*a*) Pigeons. (Cowgill)
  (*b*) Rats. (Westenbrink, Moll)
  (*c*) Dogs. (Cowgill)
V. Methods based on cure of polyneuritis
  (*a*) Pigeons. (Peters and many others)
  (*b*) Rats. (M. I. Smith)
VI. Methods based on cure of particular symptoms
  (*a*) Bradycardia in rats and pigeons. (Birch and Harris)
  (*b*) Nystagmus in rats. (Whipple and Church)
VII. Chemical methods
  (*a*) Azo test. (Kinnersley and Peters)
  (*b*) Coupling with aromatic diazo reagent. (McCollum and Prebluda)
  (*c*) Conversion to thiochrome. (Jansen-Westenbrink)
  (*d*) Precipitation methods. (Spruyt)
VIII. Methods based on behavior of plants and microorganisms
  (*a*) Yeast growth and fermentation. (R. J. Williams, Frey, *et al.*)
  (b) Growth of Phycomyes Blakesleeanus. (Schopfer)
  (*c*) Growth of cocci. (Knight)
  (*d*) Germination of seeds of higher plants. (Kögl, Bonner, Robbins)

Many of the foregoing methods have been subjected to variation as to the type of diets, use of voluntary vs. forced feeding, choice of reference standards of performance, units of activity, etc. Not infrequently there have been used methods or standards representing blends of two or more of the above-indicated tests. In some instances still more complex criteria have been followed, such as excellence of reproduction or lactation combined with growth or weight maintenance.

In considering the choice of a method of assay, one must of course be guided by many considerations having to do with the particular case. Thus, a method which may be quite satisfactory for a rich concentrate may be unsuitable for the assay of foods of low thiamin content; one which is suitable for an abundant material may be unsuitable for blood specimens which are available only in scanty amounts; procedures which are adapted to laboratories charged with a continuous program of assaying may be inconvenient or expensive for the institution which handles such matters only occasionally. Sometimes the program calls for comparison of a large number of samples and one can well wait several weeks for the final results; sometimes, as in the hospital, the result must be had within a few hours if it is to be of use. In this chapter we shall consider methods primarily from the standpoint of their serviceability in the assay of foodstuffs; secondarily

for the assay of therapeutic concentrates or synthetic products, and finally, those suitable for clinical purposes.

Whatever the particular purpose, one must judge test methods first of all on the basis of their specificity. If the criterion adopted is not a true measure of the amount of thiamin present, independent of the presence or absence of accidental or uncontrolled contaminants, it has very serious shortcomings. The same requirements as to quantitative precision will not apply to all circumstances, for in some instances one may well be justified in sacrificing accuracy to expedition or convenience or to the size and character of sample available. But as to assays of foods, it applies with great force. An estimation of thiamin intake by assay of the many components of a mixed diet is subject to so many possible errors that the most rigorous care is necessary to produce a significant result.

Reproducibility is often mistaken for accuracy or cited as evidence of specificity. A method may have high precision, that is, may be reproducible again and again with only slight variation of result, and yet be quite inaccurate because of some constant error. One may shoot unerringly and still find that he has been shooting at the wrong target.

Scanning the above methods with this feature in view and considering the problem from the standpoint of human dietaries, the methods which are based on prevention or cure of polyneuritis are superior to the others. Other dietary deficiencies may cause loss of appetite or of weight or cessation of growth. Only a deficiency of thiamin, so far as we know, can cause polyneuritis. One can but wish that polyneuritis were a more definite and easily recognizable entity and therefore must approve every effort to reduce it to objective measurement. The bradycardia method represents such an effort, but since bradycardia is not a necessary symptom of polyneuritis in all species, we must view it with reserve till the case is better established than at present. In human beriberi tachycardia is common, but bradycardia is rarely if ever encountered. While this may be due to peculiarities of the human species as compared with the rat, one cannot safely assume it without evidence. The nystagmus test is another effort to use an objective measure of polyneuritis. This, however, at the moment lacks the verification of experience in numerous laboratories. Chemical tests when fully perfected will probably prove more specific as well as more precise than any bioassay, but these methods, at least as applied to foodstuffs, are now subject to many possible errors. They are making considerable progress, but it may be some time before they can be adapted to assay of foods of very low thiamin content.

However, one must not be hypercritical. There can be no doubt that every one of the above methods has a certain validity.

Thiamin has a most pronounced effect upon appetite, growth, weight maintenance, and reproductive function of higher animals (see page 64 and following). It is likewise obviously an essential for many microorganisms as well as for the development of the embryos of higher plants. Undoubtedly most of the assays in the literature made by the various methods have a certain quantitative worth. With further development of knowledge of concurrent vitamins, nutrilites, etc., which may come into play, any of these methods may ultimately attain a satisfactory status in a quantitative sense. At present all of them must be applied with discretion (1).

Protective Methods

A somewhat more detailed description of the more important of these methods, together with a presentation of some illustrative data, is now in order. The methods based on the prevention of polyneuritis have very largely utilized birds because they show symptoms of the most characteristic sort, as was brought out in the classical work of Eijkmann. This bird method is among the simplest, least laborious, and most reliable of all methods. Satisfactory results may be obtained by a novice. It is only necessary to feed the birds on polished rice and water, together with graduated addenda of the food under test, and observe whether or not polyneuritis appears. It is much better, as well as very simple, to wash the polished rice thoroughly as was done by Jansen and Donath, whose work is discussed below. The rice is then substantially thiamin-free. Many refinements have been proposed by various workers, but they are by no means necessary for satisfactory results. One may add 5 per cent of well-autoclaved meat scrap, cod liver oil, and salt mixture to the diet to render it more adequate in other respects. For complete nutrition of fowls and pigeons, such addenda are quite necessary, but the thiamin deficiency of polished rice is so much more conspicuous than other deficiencies that polyneuritis appears before other disturbances of serious character intervene. Polished rice is so cheap and readily available as to offer great convenience over diets that have to be arduously prepared. Whole wheat, or buckwheat, brown rice or the like may be substituted for polished rice, but if this is done the grain must be thoroughly autoclaved for at least five hours at 120° C.

It has often been the practice to feed the birds forcibly in order to insure a high food intake and the prompt and more uniform occurrence of polyneuritis. It minimizes inanition, which often causes the death of birds without definite polyneuritic symptoms having been observed. There can be no doubt that forced feeding has this tendency, but it is not worth the additional labor required, especially as it introduces some uncer-

tainties. Immediate but temporary attacks of paralysis may be induced by the stuffing process.

Di Mattei (2) has proposed to supplement the usual thiamin-deficient diets with sunflower seed, which he claims prevents weight loss without preventing polyneuritis. Repetition of this work in the authors' laboratory (unpublished results) did not substantiate this conclusion. Several varieties of sunflower seed were tried and all were found to contain significant amounts of thiamin. With small addenda of sunflower seed, weight loss occurred to nearly as great an extent as without them. With large addenda, complete protection against polyneuritis was observed. The authors' experiments differed from those of di Mattei in that the birds were not forcibly fed as in di Mattei's experiments, so that the possibility cannot be excluded of a nice balance of food intake versus a low thiamin intake, such as to induce a relatively pure and uniform polyneuritis. The undoubted presence of thiamin in the sunflower seed, however, seemed to make its inclusion in the diet a questionable practice for assay purposes. We were led to suspect from di Mattei's results that sunflower seed might prove an exceptionally good source of B_3, but did not carry out the necessary experiments to answer this question.

In performing prophylactic assays of foodstuffs, pigeons are to be preferred to domestic fowls, as they develop polyneuritis somewhat more quickly; that is, in about three weeks compared with five weeks. The amount of thiamin in pure form which is required to protect pigeons against polyneuritis, if fed on washed polished rice, is believed to be about 8γ per day per bird. On unwashed polished rice it is about 4γ per day but varies considerably with the degree of polishing of the rice. The requirement for domestic fowls is not known with precision. Presumably it is higher in proportion to the food intake.

It is well to divide the birds into groups of four, or better, six. All groups are given washed polished rice *ad libitum*. One group receives no addendum, another is given daily, or every other day, a small fixed amount of the foodstuff under test, a third receives twice that addendum and so on. All addenda are administered by hand in the form of pellets in the case of solids or by pipette in the case of liquids.

The greatest disadvantage of this method is the length of time required to complete a test. Jansen and Donath introduced the use of the rice bird (3) in the same sort of test largely because this bird, being smaller, develops polyneuritis within two weeks. These authors incorporated their rice polish extract fractions in the rice. When pure thiamin was incorporated in the rice to the extent of 1γ per gram, polyneuritis was prevented. Each bird is said to require about 2γ per day to remain symptom-free. The method has been extensively used for testing foods, notably by

Van Veen (4). If these birds were readily available in Occidental countries, the method would probably be widely used. Doubtless other small birds would serve equally well, but their use has not been standardized experimentally. Sparrows have been found to be at least qualitatively suitable (5).

Arnold and Elvehjem (6, 7) have introduced the use of young chicks in quantitative determination of thiamin, using a reinforced autoclaved grain ration. The lowest level of the foodstuff under assay which is capable of protecting all the chicks from polyneuritis is determined. About 70 γ of pure vitamin per 100 grams of ration were found needed for this purpose. This permits one to state the observed antineuritic value of a foodstuff in terms of pure thiamin. The method has not yet been used by other workers, and accordingly its worth cannot be appraised precisely at present.

A method which has been considerably used in Germany is essentially identical with the rat growth method of Sherman which is described below. The standard adopted, however, is quite different. It consists in determination of the amount of the substance under assay which is required to keep the young rats alive for thirty-five days without gain or loss in weight and without display of polyneuritic symptoms (8). It requires about 6 mg. of International Standard Clay or 1.2 γ of thiamin per rat per day to produce this result.

Animal Growth Methods

Of the methods based on rate of growth, that involving the use of rats is much the most important. Most of the assays of foodstuffs which appear in the literature have been made by this method. It makes use of young rats fed upon a diet of purified foodstuffs, including carbohydrates, fat, protein, autoclaved yeast, and suitable addenda to supply the fat-soluble vitamins. Again parallel groups of animals are observed, the one upon the above-described basal diet alone, the others upon the same diet supplemented by known quantities of the foodstuff or extract under assay. If adequate thiamin is present, steady growth will occur and no polyneuritis will develop. The quantity of vitamin present is inferred from the rate of growth. Usually several trial levels of intake of the unknown are necessary to arrive at an approximately quantitative assay.

In this type of experiment there are two prevailing standards of growth which define respectively the most widely used units of B_1 content. One of these, the Chase-Sherman unit (9), is defined as the amount of B_1 per day required to produce a gain in weight of 3 grams per week during an experimental period of from four to eight weeks. The Chick-Roscoe unit is the amount of B_1

per day which produces growth at the rate of 10 to 14 grams per week over a five-week period. The International Standard Unit of B_1 is the activity of 10 milligrams of acid clay activated by contact with rice polish extract in a definite way. This amount of International Standard Clay produces growth nearly at the same rate as that adopted by Chick and Roscoe as standard (10). The rate of 10 to 14 grams is strongly preferred. This preference is based not only upon the fact that it corresponds well to that produced by the International Unit but also upon the observation that the lower standard rate of growth is marginal and that individual animals tend to deviate rather more from the mean performance at this rate as compared with higher rates. The Chick-Roscoe rate of growth is well above such a marginal rate and yet is easily attained in testing products which contain more than traces of the antineuritic vitamin. One may utilize either the Chase-Sherman diet, the Chick-Roscoe diet, or any one of a number of other diets which differ essentially from these only with respect to the nature of the carbohydrate component. This method requires meticulous care to assure an adequate amount of the various B factors other than B_1; these, in our experience, are fairly adequately supplied by yeast which has been autoclaved at *p*H 6.7 to 7.0 at fifteen pounds' pressure for six hours. This yeast should constitute 15 per cent of the diet.

In utilizing a rat growth method, careful attention must be paid to means of preventing coprophagy. This involves the use of screen-bottom cages, so that the feces do not remain accessible to the rats (11, 12, 13). It is also said to be effective to use cooked starch or dextrine as the carbohydrate component (14). This peculiar phenomenon of refection has been reliably attributed to the growth of microorganisms in the alimentary tract which are capable of synthesizing vitamin B_1 and thus providing an adequate supply of it to the host. It is discussed further in Chapter XXVIII.

It is also necessary, in utilizing this method, to have a supply of well-standardized rats and to start the experiment with them at about twenty-one or twenty-eight days of age, selecting only such animals as fall within the weight limits of 40 to 60 grams. They are placed on the B_1 deficient diet upon which they cease to grow after about two weeks. After this depletion of the body stores of thiamin, the rats are ready for the test proper. A control group is continued on the basal diet alone while other groups are given graded amounts of the foodstuff under test as an addendum to the basal diet. For a quantitative assay, especially of foods which contain relatively small amounts of the vitamin, a large number of animals in each group is necessary in order to secure precision (9, 15).

It is not satisfactory to try to interpolate or extrapolate one's

observations of the rate of growth. The experiments must be repeated at successive levels of intake until the specified rate of growth is actually approximated quite closely. The need for caution against interpolation and extrapolation is emphasized by consideration of Figure 7, taken from the work of Waterman and Ammerman (16) and illustrating the effect of graduated doses of pure crystalline vitamin upon male rats; a similar effect is noted in females. It is obvious that there exists no simple proportionality between the vitamin intake and growth rate. The inconsistencies observed in these data at levels of 1 to 5 γ of crystalline B_1 are an excellent illustration of the shortcomings of the best methods of bioassay even in the hands of workers of long experience.

The rat growth method presumes that the basal diet is perfect in all respects other than B_1. This is probably never true in practice. Experience certainly justifies the view that there are yet undiscovered or at least ill-defined components of foods which contribute in a more or less specific way to animal well-being (see Chapter X). Two of these have been termed B_4 and B_5 respectively. The evidence for their existence is not wholly convincing. Certainly neither has been adequately characterized chemically or physiologically. However, crude foodstuffs frequently cause growth responses which single pure substances fail to reveal. Accordingly many students of the subject object to any assumption that the category of vitamins at present known represents the sum total of the needs of a given species. If the basal diet in the rat growth test is not complete in every way except for its lack of thiamin, the method will give too high results for thiamin content of any addendum which contains another beneficial substance in addition to thiamin. Doubtless most of the results obtained by this method are in general substantially correct when the precautions mentioned above are rigorously observed. The reproducibility of the method is good. However, attention has recently been called to substantial differences in rates of growth of different strains of white rats (17).

Others have proposed the use of the mouse in a similar way (18). No special advantage is claimed for the mouse, but it appears to be equally well adapted to the purpose as is the rat. However, the fact that this animal has been little used for the purpose means that norms of performance are less well established for it. After a depletion period of ten to fifteen days, the mice weigh 10–12 grams. Approximately 2γ of pure thiamin daily per mouse is required to produce growth to a weight of 20–21 grams at the end of thirty-five days. It develops polyneuritis quickly and offers the further advantage as an experimental animal in that it consumes little food and requires little space for housing.

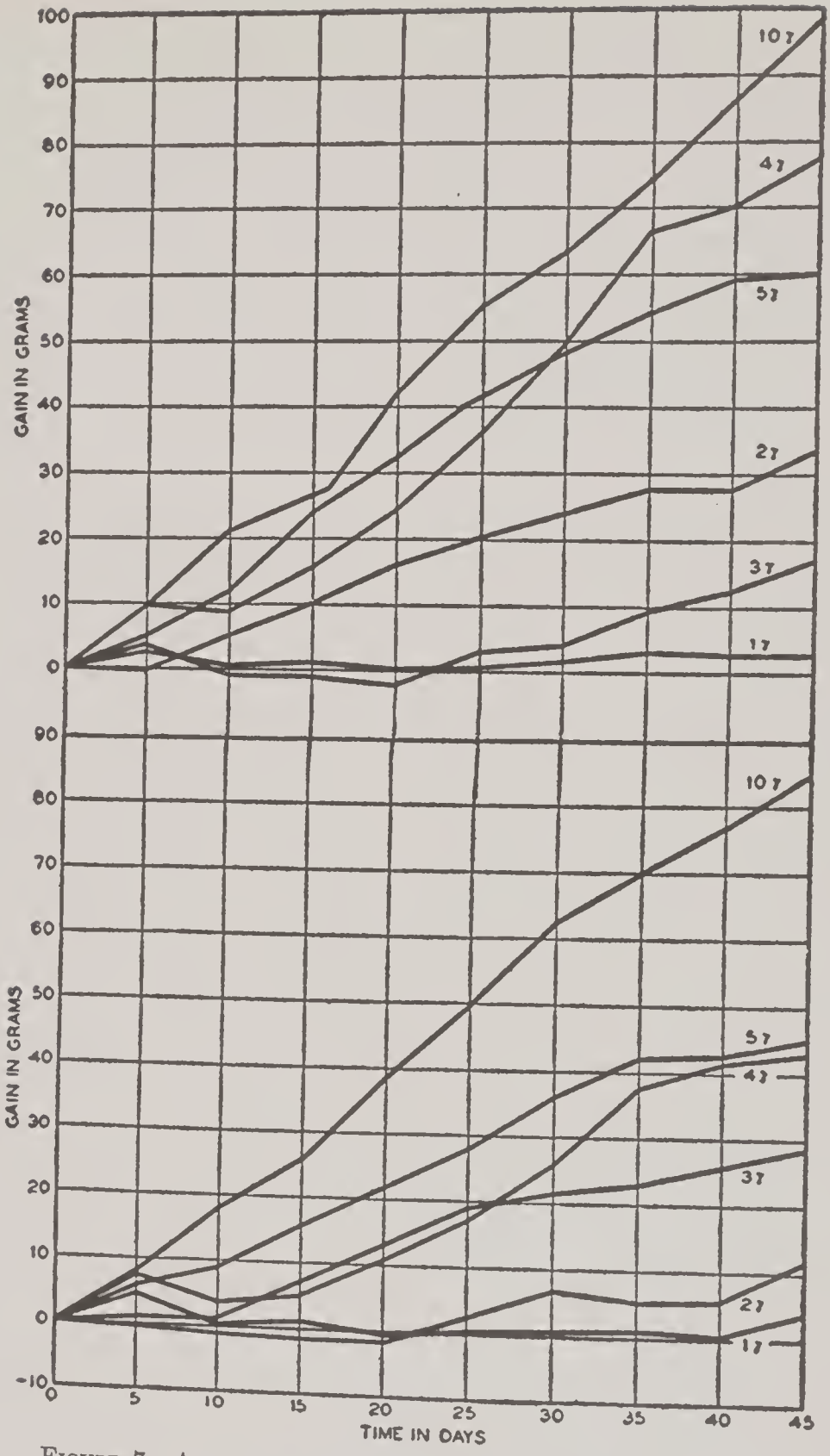


FIGURE 7. Average growth curves of rats receiving moderate amounts of crystalline vitamin B_1. The addenda were given every 5 days. The chart shows the intakes of vitamin calculated on a daily basis. The addenda were started after 28-day-old rats had been depleted 15 to 17 days on a B_1 free diet. Males above, females below. Courtesy of *Journal of Nutrition*.

Weight-Maintenance Method

This method with variations has been used by several workers, notably Williams and Seidell (19) and others, but it is not to be recommended. It appeared attractive at one time because of the possibility of observing the weight response within a few days on giving a daily dose of a thiamin-containing addendum to pigeons on a basal diet of polished rice or other equivalent food. Experience has taught, however, that the weight response is not determined solely by the thiamin given but also by the factor known as B_3 (20) which may chance to be present. B_3 cannot at present be supplied free from thiamin as an addendum to the basal diet.

There has been considerable dispute regarding this question of weight maintenance in pigeons, but the necessity for some as yet ill-defined substance which we may call the B_3 factor is well supported by later work (21, 22, 23, 24). It has been shown that while polyneuritis is prevented in pigeons by daily administration of 5 γ of the crystalline vitamin, the normal weight level cannot be attained even with addenda of 160 γ daily (see Chapter XXII). The work of Jansen and Donath, which was published prior to the discovery of B_3, also contains evidence which fully supports this view. These authors fed pigeons on polished rice with supplements of 30 to 60 γ of the pure vitamin daily and experienced marked weight losses. They repeated their experiments, supplementing the polished rice with 2.5 per cent of extracted meat powder together with salts and cod liver oil, and administered 12 to 24 γ of the vitamin daily. They still experienced a weight loss amounting to about 10 per cent of the initial weight of the birds. It is accordingly impossible to state at present the minimum B_1 which the pigeon requires for weight maintenance or how this requirement is affected by the quantity of B_3 available (25). If a supply of B_3 free from thiamin were available, this weight-maintenance method would probably be quite satisfactory, as thiamin has a profound and immediate effect on the weight curve.

Appetite and Weight-Restoration Methods

The methods under this category have a certain kinship to the animal growth methods and also to the weight-maintenance method. They resemble both in that a weight response to the administration of the substance under test is observed after a period of depletion on a thiamin-deficient diet. They differ from the growth methods in that adult animals are used and a single dose of the material under test is given rather than a succession of daily doses. They also differ from the pigeon weight-

maintenance method in that the weight response to a single dose is taken as the criterion. They have a certain merit over either in respect to rapidity of performance but are certainly not as reproducible as the rat growth method.

As applied to pigeons the basal diet may have the same general character as that used in the pigeon protective method. Preferably a diet fairly adequate in proteins, salts, fat-soluble vitamins, etc., is used. When the birds have declined some 25 to 30 per cent in weight, they are ready for use by administering a dose of the material under test. Block *et al.* (26) have used a similar method extensively, as did Seidell and Williams at one time. The difficulty is that B_3 is wanting in the diet and the response to a given dose of thiamin varies with the degree of depletion of this factor which has been attained. Grossly misleading results were often obtained when this method was used as a guide to fractionation procedures.

The appetite and weight-restoration method as applied to rats by Westenbrink (27), by Th. Moll (28), and Jansen (29) has greater merit, as in this case there are thiamin-free diets available which much more closely approach complete adequacy in other respects. The greatest difficulty is that one does not know just how much significance he should attach to the extent of rise of the weight curve on giving the test foodstuff, how much to the rapidity of the rise, and how much to the duration of the weight response. It has sometimes been proposed to divide the dose given by the number of days elapsing before the weight again falls to its former level. Moll proposes to use the area under the peak in the weight curve as the measure of thiamin activity. Doubtless a statistical treatment of a large number of observations after giving various known doses of thiamin would provide means of judging the degree of precision of each of these variants of interpretation. Such data have not yet been provided. The principal weakness of this method is the lack of assurance that the response is due solely to the thiamin in the test specimen. The method has not been sufficiently tried for the assay of foodstuffs in general to rate as reliable. Certainly it is much more expeditious and economical of animals than is the pigeon protective or rat growth method. A kindred technique has been used by Cowgill for the dog but not for routine purposes. The dog is too large for economical use.

Curative Methods

Methods of this type are the most trustworthy of all methods in our judgment because, with the possible exception of preventive methods, they are the most specific. If animals are cured of the polyneuritic symptoms developed on a high-carbohydrate

diet by a given dose of a substance, we have good ground for believing that thiamin is present. A possible exception is fat (see page 269), which has been reported to effect such cures in pigeons in some instances. Fats are, however, readily recognized and separated from the thiamin-containing portions of foodstuffs, and in any case the amount of fat required to produce a cure is large. Subsidiary deficiencies of the diet do not complicate curative tests to nearly so large an extent as methods depending on growth or weight changes. Curative tests have great advantages of convenience and speed over the protective feeding tests or growth tests. Animals may be kept for several weeks on the verge of polyneuritis by careful adjustment of the thiamin intake so that only two or three days are required to develop polyneuritis in them when they are required for test. Also the animals may be used repeatedly for successive assays. The practical advantages are shared by certain other tests, such as the weight restoration or the bradycardia test, which, however, lack an equal degree of certainty as to specificity and offer some uncertainty of interpretation in a quantitative sense. This uncertainty has to do with the relative significance of extent and duration of response which has already been mentioned.

The animal first used for curative tests was the pigeon, which develops a very characteristic polyneuritis and is very susceptible to cure. Its disadvantages are two. A considerable proportion of a group of these birds are liable to develop a progressive inanition without displaying distinctive symptoms at any time. Of those which develop these symptoms some recover spontaneously (30). It has been said that this does not occur on a polished rice diet, but in the authors' experience this is not the case. Cures have also been attributed to glucose and to heat (31).

A feature of testing associated in many minds with the pigeon curative method is the day-dose principle, whereby the dose given is divided by the number of days which elapse before recurrence of the symptoms, the quotient being called the day dose. This principle has been studied by Coward *et al., loc. cit.* It was found necessary to use a suitable empirical curve for relating the size of the dose to the duration of cure. It is clear that the duration of cure cannot be directly proportional to the size of the dose, for if this were the case a single, sufficiently large dose would prevent recurrence of symptoms for the remainder of the animal's lifetime. Ammerman and Waterman (32), working with pure thiamin injections in polyneuritic rats, showed that the larger the dose given, the poorer the utilization of it by the animal and the shorter the duration of cure in proportion to size of dose. That this must be so is evident from a study of the rapidity of rise of excretion of thiamin on giving a substantial dose (see Chapter XIX). Kinnersley and Peters (33) have

recently shown that the day-dose method does not do for the assay of pure thiamin, although they still believe that it will serve for impure concentrates. The method has some practical convenience in that a large dose is certain to save the bird, while a guess at a minimum curative dose may prove too low. We believe that a preliminary test with a large dose to ascertain approximate potency is well justified, but that for accurate assays this test should be followed by repeated trials at nearly the minimum curative dosage to ascertain the percentage of birds cured. One cannot trust too implicitly the older experience gained with crude extracts or with foodstuffs, as there are many possible complications, some of which are discussed in later pages of this chapter. Experience with pure thiamin certainly indicates that the duration of cure, while intimately related to the size of the dose, is a much less accurate measure of potency than can be obtained by repeated trial of the minimum curative dose. The extension of the day-dose principle to other test methods by Jansen (*loc. cit.*), Moll (*loc. cit.*), and by Birch and Harris (34) represents, we feel, some sacrifice of much-needed accuracy.

Because of the susceptibility of the pigeon to spontaneous cure, we much prefer the rat for curative test purposes. Our experience would lead us to recommend this test unhesitatingly as the most trustworthy of all for general purposes. The method originated with M. I. Smith (35). As adapted in minor detail by Ammerman and Waterman (32), it proved a godsend in the development of an improved method of isolation (36). It has so gained in favor in the United States that after extensive co-operative trials of many methods in twenty-five laboratories, the U. S. Pharmacopoeia Vitamin Advisory Board is now concentrating, in a further co-operative study, exclusively upon the Smith rat curative method. Five variants of the method are included in the new study, the principal difference among them being the precise composition of the diet. In general, the method is to feed young rats of about 60 grams' weight on one of the diets indicated below till polyneuritis develops, usually after about six weeks. Polyneuritis is recognized by lack of co-ordination, stiffening of legs, loss of equilibrium as shown by rolling over and over sidewise after being twirled by the tail, tremor, or almost total paralysis. Such rats are given trial doses of the substance under test either by mouth or, if of a suitable character, by injection. Intravenous injection may be used as suggested by Smith, but this requires some skill. A laboratory helper can quickly be trained to give subcutaneous injections, preferably under the skin of the back so that local reaction will not cause limb lameness which might be confused with polyneuritis. Such injection serves very well. Similar rats are treated with a

standard preparation of reference which at the present time should certainly be pure thiamin. The minimum curative dose of the test substance may be said to contain about 5 γ of thiamin. The precise standard of reference will be discussed in further detail later, but it will be well to mention here that a standard which calls for the cure of 75 per cent of all animals in a series of tests at a given dosage is much to be preferred to one calling for the cure of all animals. The latter tends to give undue weight to the occasional exceptional case.

The diets which are in use in the co-operative trial of the Pharmacopoeia Board are as follows:

Component	No. 1	No. 2	No. 3	No. 4	No. 5
Casein	18	18	16	18	18
Salt mixture	4	4	4	4	4
Autoclaved yeast	12	10	9	4	10
Vegetable oil	8	8	9 *	—	8
Cod liver oil	2	2	2	2	2
Cornstarch	56	58	60	—	58
Sucrose	—	—	—	61.25	—
Autoclaved peanuts	—	—	—	10.0	—
Purified liver extract	—	—	—	0.75	—
Raw brewers' yeast	0.3	0.5	0.1–0.5		0.4

* Butter

All diets except No. 4 are much alike. All are agreed that a small amount of raw yeast makes for a higher percentage of cases of typical polyneuritis. Unfortunately this co-operative study utilizes the International Standard Clay as a reference. The method associated with diet No. 4 involves the duration-of-cure principle which is said to be satisfactory for this diet which is designed to be complete with respect to subsidiary B factors.

Curative tests of a similar sort may, of course, be applied to other polyneuritic animals, such as the mouse or the chick, but this has been insufficiently practiced to establish norms of precision.

Bradycardia Test

This is based on the fact that rats on the usual B_1 deficient diets are subject to a marked fall in heart rate as the condition progresses toward a forthright polyneuritis. When the heart rate reaches 300 to 350, which occurs in about forty days, a single dose of the substance under test is administered by mouth or by injection. If thiamin is present, the heart rate rises promptly toward normal and declines again during the course of a few days to approximately the level at the time of dosing. The duration of the effect on heart rate is regarded as proportional to the

quantity of thiamin in the dose. Use of pure thiamin in similar tests permits conversion of the results into micrograms of thiamin. The method was proposed by Drury, Harris, and Maudsley (37) and further refined and studied by Birch and Harris (38). A statistical treatment of the precision of the method has recently been provided by Leong and Harris (39). Also a paper by Harris (40) gives an excellent résumé of the method and of a number of impressive studies which have been carried out with it, which will be discussed in other connections (see Chapters XVIII and XIX). The bradycardia is said to be unaffected by other B vitamins (41). An electrocardiograph is required, and from the testimony of those in America who have tried it, considerable skill is required to obtain reproducible results, as the degree of passivity of the animal affects the heart rate (42).

Carter and Drury extended the study to the pigeon, which exhibits a heart block as well as bradycardia (43). Carter (44) once ascribed the heart block to some other factor than B_1, as it was not cured by doses sufficient to clear up polyneuritis. Essentially the bradycardia method above outlined has been utilized for the study of pigeons (45).

In spite of the extended series of apparently successful applications of the method, it seems that its specificity should be tested more rigorously than has yet been done. There can be no question that pure thiamin has the effect of raising the heart rate of rats in proportion to the quantity administered. However, it has not been shown that the heart rate is independent of the character of the diet or of the food under test. Birch and Mann (46) reported a B_1 free coenzyme which seemed to show some curative effect upon rat bradycardia, and Birch and Mapson (47) found that adenosine or adenylic acid from muscle or yeast accentuates the bradycardia. Such effects may not interfere with the test for many purposes, but for the assay of foodstuffs which have to be given in large amounts to effect a cure because of their low thiamin content, such secondary influences might well introduce serious discrepancies. For example, if adenine compounds are effective by mouth, the relative amount of such compounds in the test food as compared with that in the basal diet might be significant, especially as the autoclaved yeast contained in most diets is unusually high in such compounds. In a similar way the proportions of the chief components of the test food might influence the result. This sort of difficulty is rendered the more probable by the observations of Parade (48), who has contributed a very serious criticism of the bradycardia method. According to his experiments, which appear to be sound, bradycardia in rats is induced merely by restricting the amount of food offered, even when the food has a quite adequate thiamin content. He con-

siders it a consequence of inanition rather than of thiamin deficiency *per se*. It parallels the fall of body temperature and the declining weight curve. The surprisingly high thiamin contents of leaf and root vegetables reported by Baker and Wright might be due to such a cause. At all events the whole history of vitamin testing has proven so full of pitfalls that utmost care should be taken to exclude such possibilities before the literature is filled with results that may have to be revised. One should at least make sure that a variety of thiamin-deficient diets all lead to substantially the same degree of bradycardia and that it is equally curable by pure thiamin. Sampson (50) has called attention to the fact that the drop in heart rate with age in normal rats between twenty-eight and fifty-six days of age is quite sufficient to introduce a sensible error. He has also noted that thiamin given in the form of International Standard Clay results in a lower heart rate than is the case if pure thiamin is given in an amount sufficient to produce equal growth.

GROWTH RATES OF RATS ON CHASE-SHERMAN DIET WITH B_1 ADDENDA
(Kindness of Mr. W. L. Sampson)

DAILY ADDENDUM	GROWTH IN 28 DAYS, GRAMS	HEART RATE AT END OF PERIOD
3.5 γ thiamin	26.9	429
4.0 γ thiamin	26.3	429
4.5 γ thiamin	31.3	454
10 mg. International Standard	28.5	395
3.5 γ thiamin	20.8	418
4.0 γ thiamin	26.8	432
10 mg. International Standard	26.3	397
4.0 γ thiamin		422
10 mg. International Standard		372

Such deviations might be due to any one of several causes. The clay supplies thiamin in less available form, but it also supplies some other little-known B factors which may favorably influence growth but fail to raise the heart rate, or the clay may carry impurities which unfavorably influence the heart rate.

These comments are offered for future betterment of the method. Such shortcomings cannot markedly influence the relative values of thiamin content obtained in the examination of a series of samples of essentially similar material: *e.g.*, urine specimens. The results of such assays which have already been published are of inestimable value. Once the equipment is made available and the technique mastered, it is evident that the method permits rapid and convenient assays.

Church (51) has proposed a vestibular function test on rats

as a gauge of antineuritic potency. It depends on the fact that if rats are rotated in a horizontal position with the head at the center of rotation, movements of the eyes are induced (nystagmus). The duration of nystagmus is prolonged in B_1 deficiency but is normalized by administering the vitamin. It has not been subjected to systematic trial for assay purposes, and need not be discussed further.

Units and Standards

Before proceeding to the discussion of chemical methods of assay of thiamin, it will be well to discuss the matter of units and standards. These were devised, prior to the availability of pure thiamin, as a means of expressing the antineuritic potency of foodstuffs, concentrates, etc., in some common terms. In early times the antineuritic value of a food was sometimes expressed in terms of equivalence to a given weight of rice polish or dry yeast. These standards were unsatisfactory because the standard materials vary in activity from specimen to specimen. To remedy this, there was adopted (52) an International Standard consisting of acid clay adsorbate of vitamin B_1 from rice polish extract, made in a fairly well defined manner, essentially according to the method used by Jansen and Donath as a first step in their isolation procedure. An International Unit was defined as the antineuritic activity of 10 milligrams of this adsorbate.

This particular choice has in the past two or three years proven to be very unfortunate from one standpoint in that the thiamin which it contains is not always (perhaps never) fully and promptly released in the digestive tract of experimental animals. Healthy rats appear to be able to elute the thiamin from the clay fairly successfully, but as they approach the polyneuritic state they do so less and less efficiently. On this account, the supposedly Standard Unit has a larger value in protective tests than in curative ones. Sampson and Keresztesy (53) found that 20 milligrams of clay each, given by mouth, cured seventeen out of twenty-five polyneuritic rats, or 68 per cent. When the clay was extracted with quinine sulfate according to the method of Williams *et al.*, the extract of 10 milligrams of the clay was sufficient to cure the rats to the extent of fifteen out of twenty cases, or 75 per cent. In another experiment, these authors treated a portion of a pure thiamin solution with fuller's earth, which is essentially similar to the clay used for preparing the International Standard. The clay was filtered off and dried. When tested on polyneuritic rats, it was found necessary to give 14 milligrams of this adsorbate to cure 73 per cent of the polyneuritic rats, whereas the quinine extract of 9.16 milligrams of it had been sufficient to cure an equal percentage (54).

Once this fact was established a review of the literature clearly revealed gross discrepancies which could be explained by the simple assumption that the efficiency of elution of the vitamin in the alimentary tract varies with the condition of the animals. Thus Smith and Seidell (55) reported that 20 milligrams of International Standard represented the minimum curative dose for polyneuritic rats without stating how many animals were used or what percentage were cured, although 100 per cent is implied. Roscoe (56) found in the case of rats that 10 milligrams of the clay cured 30 per cent; 20 milligrams, 60 per cent; 30 milligrams, 80 per cent; ten rats being used for each test. The former authors conclude that one International Unit equals 3 γ of pure thiamin; the latter did not make an estimate in terms of the pure substance. However, Mr. W. L. Sampson has kindly supplied us with the following further results of curative tests on polyneuritic rats:

Comparison of International Standard Adsorbate and Thiamin Chloride by Mouth

Material	Dose	Number of Rats	Number Cured	Per Cent Cured
International Standard	18 mg.	18	7	38.8
International Standard	20 mg.	25	17	68.0
International Standard	22 mg.	25	20	80.0
Thiamin	4 γ	38	20	52.6
Thiamin	5 γ	55	40	72.7
Thiamin	6 γ	30	25	83.3

These results indicate that one I.U. is equivalent to 2.5 γ of thiamin or slightly less in rat curative tests when the Standard is given as such. However, when the International is first eluted and the eluate is compared with the pure thiamin chloride, 10 milligrams of the clay gives nearly as much response as 5 γ of thiamin as seen below:

Comparison of Eluate of International Standard with Thiamin Chloride by Injection

Material	Dose	Number of Rats	Number Cured	Per Cent Cured
International Standard *	8 mg.	18	4	22.2
International Standard	10 mg.	30	18	60.0
International Standard	12 mg.	18	13	72.0
Crystalline B_1	4 γ	21	9	42.6
Crystalline B_1	5 γ	45	29	64.4
Crystalline B_1	6 γ	27	19	70.0

* In this assay the International Standard clay was eluted with quinine sulfate according to the method of Williams (57) and the eluate so obtained administered to the rats by subcutaneous injection.

In the rat growth test, Mr. Sampson has found that 4 γ of thiamin produce the same growth as 1 I.U., while Ammerman and Waterman (16) in less exhaustive trials rated 1 I.U. = 5 γ of thiamin for rat-growth purposes.

Coward, Burn, Ling, and Morgan (1), in one of the most critical papers that has ever appeared on methods of B_1 assay, reported that 15 milligrams of clay cured 25 per cent of polyneuritic pigeons; 30 milligrams cured 47 per cent; 60 milligrams cured 80 per cent. Waterman and Ammerman (24) stated that 3 γ of pure thiamin always brought about some improvement; 4 γ was generally curative, while 5 γ never failed to cure. The latter is in fair agreement with Ohdake and Yamagishi, who found that 3 γ of the pure vitamin was curative. If we regard 60 milligrams of the clay as the minimum curative dose for the pigeon as per Coward's results, we have 1 I.U. = $\frac{4}{6}$ = 0.67 γ of pure thiamin. Kinnersley and Peters (33), using the day-dose method on pigeons, arrive at the conclusion that 1 I.U. = 2 γ of thiamin. These authors record sixty-six polyneuritic birds which over a period of years were dosed with 50 milligrams each of Jansen's activated clay. Of these only three are recorded as not cured. This is more favorable than the percentage cured in the experiments of Coward *et al.*, but at all events confirms the idea that even 50 milligrams of the Standard do not always cure. It is small wonder that there has been difficulty in converting one worker's results into terms of another and in settling such questions as the respective requirements of different species or the potency of foodstuffs in terms of International Units.

When rats are in an intermediate state between health and polyneuritis, one might suppose that an intermediate efficiency in eluting the vitamin from the clay would be observed. For the purposes of the bradycardia test and the weight-restoration test, rats are used in this intermediate condition and accordingly we find Leong and Harris (39) reporting 1 I.U. = 2.9 γ and Moll (28), whose test involves the duration-of-cure principle as does Peters' pigeon test, arrives at 1 I.U. = 2 γ. Jansen, however, using an essentially similar method, concluded that 1 I.U. = 3 γ (29). For convenience, the conversion values which the authors regard as most accurate are summarized:

AVAILABLE THIAMIN IN TEN MILLIGRAMS INTERNATIONAL STANDARD CLAY

Quinine extraction	4–5 γ
Rat growth	4
Rat curative	2.0–2.5
Rat weight restoration	2.0–3.0
Rat bradycardia	2.9
Pigeon curative (day dose)	2 *
Pigeon (percentage cured)	0.7 *
Pigeon protective †	4.0

* These values have little practical value, since the duration of cure in pigeons has been reported as not proportional to the dose of pure thiamin and since the "percentage cured" form of statement has been little used with pigeons.

† Unwashed polished rice is assumed as basal diet (59).

Some other units which have been used should be mentioned. The Chick-Roscoe unit of rat growth is very nearly equal to 4 γ of thiamin; the Sherman-Chase unit to 1.5 γ, or perhaps 2 γ. The Cowgill milligram equivalent may be taken as equal to about 0.2 γ. The rat curative unit is 5 γ on the basis of 70 per cent of animals cured; 6 to 7 γ on the basis of practically all animals being cured.

There has been a great desire to adopt some standard factor for converting International Units to weight of thiamin for commercial purposes and also, by a strange perversity of the human mind, for converting grams of thiamin to International Units. As a compromise a value of 300,000 I.U. per gram of thiamin is coming to be widely accepted and used by drug firms in the United States, while Continental Europe tends to adhere to the value of 500,000 I.U. per gram of thiamin. The former is certainly the better value of the two. The preferred course according to our view is to convert I.U. to grams, or more conveniently to micrograms (γ), by using factors appropriate to the method used in making the assay. This is the method followed in calculating the distribution of thiamin in foods in the next chapter.

A move is now under way by the International Conference on Vitamin Standardization to adopt pure thiamin as the standard. It will be very helpful if this is done. See page 169. It is also to be hoped that the International Unit will be abandoned rather than merely revised. Since it is customary to express quantity of all chemical substances in the usual units of weight, such as the gram, the perpetuation of an arbitrary unit for thiamin is quite unnecessary. Conversion of quantities of a pure substance into equivalence to a crude preparation containing no more than 0.05 per cent of the active constituent in admixture with a great deal of inert material and small amounts of other physiologically active substances is merely a temporary absurdity into which we have been led by the current of events.

Chemical Methods of Assay

The formaldehyde-azo test, a modification of the Pauly reaction, whereby a red color is produced by adding diazotized sulfanilic acid to a solution containing thiamin in the presence of formaldehyde, has been proposed for purposes of assay (60). The purpose of the formaldehyde is to stabilize the color which otherwise fades by oxidation. In the presence of other reducing agents, as, for example, the reducing substances often present in urine, the formaldehyde may be unnecessary. The method has been declared serviceable in the later stages of fractionation of food extracts but has not been adapted to crude materials.

A very kindred color reaction has been proposed by Prebluda and McCollum (61). It utilizes diazotized p-amino acetanilide or p-amino acetophenone for coupling to form a red dye which, being soluble in immiscible solvents, may be extracted from the aqueous suspension or solution and estimated colorimetrically. The method is still under process of development.

Another chemical method which has advanced further in development depends on the conversion of thiamin to thiochrome and the estimation of the latter by its fluorescence. It was proposed by Jansen (62), who used a solution of quinine as a standard of comparison. A fluorometer was used for the comparison. Karrer and Kubli (63) utilized as a standard of reference a pure solution of thiamin which was oxidized with ferricyanide to thiochrome in parallel with the oxidation of the unknown. Westenbrink and Goudsmit (64) proposed a number of expedients to adapt the method to the assay of urine. Among these was the use of an atmosphere of nitrogen to avoid oxidative effects of air. Another improvement was the use of franconite to adsorb thiamin from the urine and thus separate it from interfering substances and to carry out the oxidation with ferricyanide in a nitrogen atmosphere. The thiochrome formed is extracted from the reaction solution with isobutanol, and the intensity of the blue fluorescent light is measured with a Cohen fluorometer.

Further modifications of the thiochrome method have been made by Cline and Jolliffe (65) which may bring it within the range of practicality for the clinical laboratory. Among the principal improvements is the use of a solution of pure thiochrome as a standard of comparison in lieu of the extracted product of a thiamin oxidation as proposed by Westenbrink and Goudsmit. Pure thiochrome is now commercially available [1] for this purpose and its use affords added assurance that the standard of reference will be constant. Cline also has substituted artificial zeolite [2] for franconite as an adsorbent. This has two advantages: namely, that yellow coloring matters and reducing substances of the urine are little adsorbed by it. In consequence of the relative absence of the coloring matters in the eluate one obtains the final thiochrome in such form that its fluorescence in ultraviolet light can be compared with that of the standard solution by eye in a very simple color comparator. This avoids the necessity for relatively expensive photoelectric apparatus for measuring fluorescence quantitatively. The absence of the reducing substances in the eluate eliminates the need for many preliminary trials to ascertain the proper amount of ferricyanide to use for oxidation. As pointed out by the Dutch workers, either an excess

[1] From Merck and Company, Rahway, N.J.
[2] Obtained from Permutit Company, N.Y., as 10–40-Decalso.

or a deficiency of ferricyanide results in a low value of thiamin content. About 66 to 98 per cent conversion to thiochrome of thiamin added to urine is achieved. Somewhat better control is needed to insure complete adsorption of thiamin without an excess of foreign substances. Oxidation is conducted in nitrogen atmosphere as previously suggested. Thiamin in pure water can be determined with high precision by this method.

The method has been little used for foods as yet and has been checked for this purpose only against the bradycardia method (66).

Before seriously undertaking the accurate assay of foods by chemical methods, it will be necessary to study very carefully possible methods for extracting thiamin quantitatively from foods. We have used acidulated methyl or ethyl alcohol in a continuous type of extractor with promising results in the case of a few foodstuffs: viz., whole wheat, rice polish, orange and milk powder. Indeed, with rice polish we have had an extensive and very satisfactory experience. Extraction of this materal for sixteen to twenty hours apparently gives complete extraction, for further extraction yields no activity. Further the assay of the original rice polish by feeding to polyneuritic rats checks very well with similar assay of the extract. The details of the method as applied to potato, cabbage, and lettuce follow:

Potatoes (1434 grams) were boiled with the skins on for 25 minutes. The water (1300 cc.), *p*H 7, was drained off and treated with 7.2 grams of fuller's earth, which was then filtered off and dried.

The cooked potatoes (1371 grams), after peeling, were macerated for 24 hours in boiling methyl alcohol containing 1 per cent acetic acid. The mass was filtered on a Buchner funnel and the solids were dried at room temperature before a fan and extracted in a large thimble continuously with a new charge of acid solvent in a hot vapor extractor for 2 hours. The extraction was repeated twice with new charges of solvent for 4 and 14 hours, respectively. All the extracts, including the original filtrate, were combined and treated with 7.2 grams of fuller's earth.

1548 grams of cabbage and 1353 grams of lettuce were each treated similarly except that the cooking in water was omitted in the case of lettuce.

Each of the five lots of adsorbates were separately eluted with quinine acid sulfate according to the previously recorded method (36). The quinine-free eluates were concentrated *in vacuo* to volumes suitable for injection into polyneuritic rats. The results were:

	Grams Food Required to Yield Dose	*Number of Rats*	*Number Cured*
Potato water	100	8	0
Potatoes	7.5	8	6
Cabbage water	not tested	—	—
Cabbage	40	5	0
	60	2	2
Lettuce	30	8	6

The most troublesome part of the process is the desiccation of the food with alcohol. Unless this is carried out thoroughly, thimbles of excessively large volume will be required for the later extractions. When properly desiccated, the above lot of cabbage weighed only 42 grams and could be placed in a commercially available extraction apparatus.

For molasses, milk, and other fluids containing little filterable solids, it is only necessary to dilute, treat with fuller's earth, and proceed as above.

For purposes of chemical determination of thiamin it will probably be necessary to remove the thiamin from the extract by an adsorbent and elute the thiamin therefrom before applying oxidative or coupling reactions. Without some such preliminary partial isolation of the activity, great difficulty will be encountered with interfering substances which will react with the reagents, impair the color standards, etc. As adsorbents, zeolite and franconite (64) offer great promise on account of the ease with which thiamin may be eluted from them with mild reagents.

There seems to be no doubt that many of the animal assay methods which have been described in this chapter would be greatly improved for purposes of food assays by using a more or less refined extract of the food instead of the gross food itself. Thus in a rat growth test of a foodstuff, such as bread containing perhaps 0.5 γ of thiamin per gram, it becomes necessary to give a daily addendum of 8 grams of the bread to achieve the standard rate of growth. The regimen is entirely upset, as the basal diet is largely replaced by the addendum. Far too many unaccountable results of slight variations in the diet are recorded in the literature to permit one to have much confidence in his judgment as to whether or not the result will be affected by the secondary consequences of dietary alterations. A good example of unpredictable effects is given by Schrader and Prickett (70), who observed that dextrinized cornstarch in the diet was far less favorable to development of polyneuritis in rats than raw cornstarch. There is great need for more critical application of chemical methods, either by themselves or more particularly in association with biological methods of assay. A year's solid work in this field by a group of three or four properly trained individuals might well revolutionize our present knowledge of the distribution of thiamin in foods and alter our perspective of many dis-

puted phases of its role in nutrition. However, the fact that thiamin occurs in foods only in the proportion of five to forty parts per ten million and that it is very sensitive to certain reagents should not be ignored as the basis of need for great care in the work.

Growth of Microorganisms

Assay methods of this type have attracted attention for many years, primarily, no doubt, because the short duration of the life cycle of microscopic organisms favors the development of a rapid test. Such a suggestion was first made by R. J. Williams (71) and also by Bachmann (72), who noted that growth of yeast was promoted by extracts of antineuritic foods. Schopfer and Jung (73) have recently reviewed the possibilities of using a mold *Phycomyces Blakesleeanus* for thiamin assays. This subject has been explored extensively for years by Schopfer and associates, and this organism is better known than any other in regard to its thiamin requirement. The idea of using yeast for the purpose has been revived recently by Schultz, Atkin, and Frey (74). We are, however, in full accord with R. J. Williams' belief that such methods are extremely hazardous (75). Williams and Roehm (76) and Williams and Saunders (77) showed that while vitamin B_1 had a very potent influence on the growth of certain yeasts, other nutrilites had even more profound influence. Further, as can be seen by reference to Chapter XXVIII, intermediates of thiamin or kindred substances can be used as substitutes for thiamin by various microorganisms as well as by the roots of higher plants. Certain Phytophora require the vitamin itself, but their use for test purposes has not been studied. In view of the fact that animals cannot use substitutes, misleading results would sometimes be obtained by the use of such organisms for assay purposes. Especially in the case of cooked foods, there is considerable probability of the presence of certain amounts of the cleavage products resulting from thermal decomposition.

However, for certain special purposes such tests may be valuable. Meiklejohn has used the Phycomyces test for determination of thiamin in blood. In such a case there is little choice of method because only a minute sample is available. Moreover, in such a complex biological fluid there is much less probability of the existence of subsidiary deficiencies which might affect the growth of the organism and one can run check tests of the culture medium with and without small amounts of added thiamin, also with and without added blood, to verify the results. However, a growth period of ten days is necessary, and the method seems more suitable for research purposes than for the clinical laboratory.

Summary

Most of the methods in use for assay of thiamin in foods and concentrates have been reviewed. Of these methods, those based on prevention or cure of polyneuritis are preferred as most specific and trustworthy. The rat curative test is regarded as combining as great a precision as is offered by any other method with reasonable convenience and rapidity.

The rat growth method shows a good degree of reproducibility but its specificity in a precise quantitative sense is subject to some question. The bradycardia method is more doubtful, as is also the case with weight restoration and maintenance methods.

The day-dose principle as applied to all methods is regarded as a sacrifice of precision.

The International Standard unit of antineuritic activity has not a fixed value but varies with the type of test. For curative tests 1 I.U. = 1 to 2 γ of pure thiamin chloride; for protective tests, 4 γ; for tests using animals partially depleted of thiamin but not yet polyneuritic, 1 I.U. has an intermediate value. The best compromise value, when compromise is necessary, is 1 I.U. = 3.3 γ thiamin chloride.

Chemical methods of assay are promising but as yet insufficiently developed for the study of foods; those involving growth of microorganisms require more rigorous proof of specificity before they can be trusted for general purposes.

BIBLIOGRAPHY

1. Coward, K. H., Burn, J. H., Ling, H. W., and Morgan, B. C. E.: The Determination of the Antineuritic Vitamin. Biochem. J. *27*, 1719, 1933.
2. Di Mattei, P.: La dieta dei semi di girasole per lo studio del beriberi aviario sperimentale. Arch. di fisiolog. *25*, 56, 1927.
3. Jansen, B. C. P., and Donath, W. F.: Isolation of the Antineuritic Vitamin. Mededeel. v. d. Dienst. Volksgezondh. Ned. Ind. *16*, 186, 1927.
4. Van Veen, A. G.: Het B_1 gehalte der voornaamste Indische voedingsmiddelen. Geneeskund. Tijdschr. Ned. Ind. *75*, 2050, 1935.
5. Vedder, E. B.: Private communication.
6. Arnold, A., and Elvehjem, C. A.: Studies on the Quantitative Estimation of Vitamin B_1. Rochester Meeting Amer. Chem. Soc. Sept. 8, 1937.

7. Elvehjem, C. A.: Vitamin B_1 Assay by Preventive Biological Test, Applicable to Any Feedingstuff Containing Vitamin B_1. Jour. Assn. Official Agric. Chemists *18*, 354, 1935.
8. Scheunert, A., and Schieblich, M.: Stability of International B_1 Standards. Proc. 5th Intern. Cong. Tech. Chem. Agric. Ind. Holland I, 13, 1937.
9. Chase, E. F., and Sherman, H. C.: Quantitative Study of the Determination of the Antineuritic Vitamin B. Jour. Amer. Chem. Soc. *53*, 3506, 1931.
10. Chick, H., and Roscoe, M. H.: A Method for the Assay of the Antineuritic Vitamin B_1 in Which the Growth of Young Rats Is Used as a Criterion. Biochem. J. *23*, 498, 1929.
11. Steenbock, H., Sell, M. T., and Nelson, E. M.: Modified Technique in the Use of the Rat for Determination of Vitamin B. Jour. Biol. Chem. *55*, 399, 1923.
12. Salmon, W. D.: Vitamin B in the Excreta of Rats on a Diet Low in This Factor. Jour. Biol. Chem. *65*, 457–62, 1925.
13. Fridericia, L. S., Freudenthal, P., Gudjonnson, S., Johansen, G., and Schoubye, N.: Refection, a Transmissible Change in the Intestinal Content, Enabling Rats to Grow and Thrive without B Vitamin in the Food. Jour. Hyg. *27*, 70, 1927.
14. Roscoe, M. H.: Spontaneous Cures in Rats Reared upon a Diet Devoid of Vitamin B and Antineuritic Vitamin. Jour. Hyg. *27*, 103, 1927.
15. Sherman, H. C., and MacArthur, E. H.: A Quantitative Study of the Determination of Vitamin B. Jour. Biol. Chem. *74*, 107–115, 1927.
16. Waterman, R. E., and Ammerman, M.: Studies of Crystalline Vitamin B. Effect of Graduated Doses on Growing Rats. Jour. Nutrition *10*, 35, 1935.
17. Light, R. F., and Cracas, L. J.: Vitamin B_1 Requirement of Different Strains of White Rats. Science *87*, 90, 1938.
18. Cerecedo, L. R., Hennessy, D. J., Kaszuba, F. J., and Thornton, J. J.: The Vitamin B_1 Requirement of the Albino Mouse. Meeting of the Amer. Chem. Soc., Chapel Hill, N. C., April 1937.
19. Williams, R. R., and Seidell, A.: The Chemical Nature of the "Vitamins." II. Isomerism in Natural Antineuritic Substances. Jour. Biol. Chem. *26*, 431–456, 1916.
20. Williams, R. R., and Waterman, R. E.: The Tripartite Nature of Vitamin B. Jour. Biol. Chem. *78*, 311–322, 1928.
21. Carter, C. W., Kinnersley, H. W., and Peters, R. A.: Maintenance Nutrition in Adult Pigeon; Relation to Torulin (Vitamin B_1). Biochem. J. *24*, 1832 & 1844, 1930.
22. O'Brien, J. R.: Vitamin B_3. Biochem. J. *28*, 926, 1934.
23. Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon. The Influence of Dietary Protein and Vitamin B_3. Biochem. J. *29*, 2746, 1935. Maintenance Nutrition in the Pigeon. Vitamin B Concentrates. Biochem. J. *30*, 43, 1936.

24. Waterman, R. E., and Ammerman, M.: Studies of Crystalline Vitamin B. VI. The Effect of Graduated Doses upon Pigeons. Jour. Nutrition *10*, 161, 1935.
25. Carter, C. W., and O'Brien, J. R.: Maintenance Nutrition in the Pigeon. The Effect of Vitamin B_1. Biochem. J. *31*, 2264, 1937.
26. Block, R. J., Cowgill, G. R., and Klotz, B. H.: The Antineuritic Vitamin. I. The Method of Assay, Concentration of the Vitamin with Silver under Various Conditions and Its Solubility in Certain Organic Solvents. Jour. Biol. Chem. *94*, 765, 1932.
27. Westenbrink, H. G. K.: Über den Gehalt an Vitamin B_1 der Organe von weissen Ratten bei normaler und vitamin-B_1-freier Ernährung. Arch. Neerland. Physiol. *17*, 562, 1932.
28. Moll, Th.: Beitrag zur biologischen Prüfung des Vitamin B_1. E. Merck Jahresber. *49*, 57, 1935.
29. Jansen, B. C. P.: Wertbestimmung des Völkerbund-Standart Präparats für Vitamin B_1. Zeit. f. Vitaminforsch. *5*, 254, 1936.
30. Kon, S. K., and Drummond, J. C.: The Physiological Role of Vitamin B. Part III. Study of Vitamin B Deficiency in Pigeons. Biochem. J. *21*, 632, 1927.
31. Kinnersley, H. W., Peters, R. A., and Reader, V.: Antineuritic Concentrates. III. The Curative Pigeon Test. A Critique. Biochem. J. *22*, 276, 1928.
32. Waterman, R. E., and Ammerman, M.: Studies of Crystalline Vitamin B. IV. Injection Method of Assay. Jour. Nutrition *10*, 25, 1935.
33. Kinnersley, H. W., and Peters, R. A.: Crystalline Torulin (Vitamin B_1) and the International Vitamin B_1 Standard. Biochem. J. *30*, 985, 1936.
34. Birch, T. W., and Harris, L. J.: Bradycardia in Vitamin B_1-deficient Rat and Its Use in Vitamin B_1 Determinations. Biochem. J. *28*, 602, 1934.
35. Smith, M. I.: A New Method of Evaluating the Potency of Antineuritic Concentrates. U. S. Public Health Rept. *45*, 116, 1930.
36. Williams, R. R., Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin. Jour. Amer. Chem. Soc. *56*, 1187, 1934.
37. Drury, A. N., Harris, L. J., and Maudsley, C.: Vitamin B Deficiency in the Rat. Bradycardia as a Distinctive Feature. Biochem. J. *24*, 1632, 1930.
38. Birch, T. W., and Harris, L. J.: Bradycardia in the Vitamin B_1-deficient Rat and Its Use in Vitamin B_1 Determinations. Biochem. J. *28*, 602, 1934.
39. Leong, P. C., and Harris, L. J.: Antineuritic Potency of Synthetic and Natural Crystalline Vitamin B_1 as Determined by the Bradycardia Method. Biochem. J. *31*, 672, 1937.
40. Harris, L. J.: La Détermination de Vitamine B_1 par la Methode "Bradycardia." V^e Cong. Inter. Tech. Chim. Indust. Agri. Schéveningue. Section 1, p. 100, 1937.

41. Drury, A. N., and Harris, L. J.: Vitamin B Deficiency in the Rat, Bradycardia as a Distinctive Feature. Chem. Ind. *49*, 851, 1930.
42. Robertson, E. C., and Doyle, M. E.: Difficulties in the Use of Brachycardia Method of Assaying Vitamin B_1. Proc. Soc. Exp. Biol. Med. *37*, 139, 1938.
43. Carter, C. W., and Drury, A. N.: Heart Block in Rice-Fed Pigeons. J. Physiol. *68*, Proc. 1, 1929.
44. Carter, C. W.: Heart Block in Pigeons, Curative Factor. Biochem. J. *24*, 1811, 1930.
45. Sankaran, G., and Krishman, B. G.: Heart Rate in B_1 Deficiency. Indian Jour. Med. Res. *23*, 747, 1936.
46. Birch, T. W., and Mann, P. J. C.: Activation of Lactic Dehydrogenase and Its Relation to Role of Vitamin B_1. Biochem. J. *28*, 622, 1934.
47. Birch, T. W., and Mapson, L. W.: Role of Adenylic Acid in Vitamin B_1 Deficiency. Nature *138*, 27, 1936.
48. Parade, G. W.: Relationship between B_1 Deficiency and Bradycardia. Zeit. f. Vitaminforsch. *6*, 327, 1937.
49. Baker, A. Z., and Wright, M. D.: The Vitamin B_1 Content of Foods. Biochem. J. *29*, 1802, 1935.
50. Sampson, W. L.: Private communication.
51. Church, C. F.: Functional Studies of the Nervous System in Experimental Beriberi. Amer. Jour. Physiol. *111*, 660, 1935.
52. League of Nations—Health Organization Report: The Antineuritic Vitamin B. London, June 23, 1931, p. 75.
53. Sampson, W. L., and Keresztesy, J. C.: A Comparison of Some Methods for the Extraction of Vitamin B_1 from International Standard Acid Clay. Proc. Soc. Exp. Biol. Med. *36*, 30, 1937.
54. Keresztesy, J. C., and Sampson, W. L.: Utilization of Vitamin B_1 from Fuller's Earth Adsorbates. Proc. Soc. Exp. Biol. Med. *36*, 686, 1937.
55. Smith, M. I., and Seidell, A.: The Preparation of a Concentrate of Vitamin B_1 and B_2 from Brewers' Yeast. Public Health Reports *51*, 685, 1936.
56. Roscoe, M. H.: B Vitamins in Human Urine. Biochem. J. *30*, 1053, 1936.
57. Williams, R. R., Waterman, R. E., and Keresztesy, J. C.: Larger Yields of Crystalline Antineuritic Vitamin. Jour. Amer. Chem. Soc. *56*, 1187, 1934.
58. Coward, K. H., Burn, J. H., Ling, H. W., and Morgan, B. G. E.: The Determination of the Antineuritic Vitamin. Biochem. Jour. *27*, 1719, 1933.
59. Ohdake, S., and Yamagishi, T.: Oryzanin, "Antineuritic Vitamin." V. On the Activity of Oryzanin Hydrochloride. Bull. Agric. Chem. Soc. Japan *11*, 111, 1935.
60. Kinnersley, H. W., and Peters, R. A.: The Formaldehyde-azotest for Vitamin B_1. Biochem. J. *28*, 667, 1934.

61. Prebluda, H. P., and McCollum, E. V.: A Chemical Reagent for the Detection and Estimation of Vitamin B_1. Science *84*, 488, 1936.
62. Jansen, B. C. P.: A Chemical Determination of Aneurin (= Vitamin B_1) by the Thiochrome Reaction. Rec. Trav. Chim. Pays Bas *55*, 1046, 1936.
63. Karrer, W., and Kubli, V.: Determination of Vitamin B_1 (Aneurin). Helv. Chim. Acta *30*, 369, 1937.
64. Westenbrink, H. G. K., and Goudsmit, J.: Determination of Aneurin (Vitamin-B_1) in Urine by the Thiochrome Reaction. Rec. Trav. Chim. Pays Bas *56*, 803, 1937.
65. Cline, J. K., and Jolliffe, N.: Chemical Estimation of Thiamin (Vitamin B_1) in Urine. J. Biol. Chem. (In Press.)
66. Pyke, M. A.: The Chemical Measurement of Vitamin B_1 in Foodstuffs and Biological Material by Means of the Thiochrome Reaction. Biochem. J. *31*, 1958, 1937.
67. Cerecedo, L. R., and Hennessy, D. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. I. Rice Polishings. Jour. Amer. Chem. Soc. *59*, 1617, 1937.
68. Cerecedo, L. R., and Kaszuba, F. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. II. Brewers' Yeast. Jour. Amer. Chem. Soc. *59*, 1619, 1937.
69. Cerecedo, L. R., and Thornton, J. J.: Synthetic Zeolites in the Isolation of Vitamin B_1. III. Wheat Germ. Jour. Amer. Chem. Soc. *59*, 1621, 1937.
70. Schrader, G. A., and Prickett, C. O.: Jour. of Nutrition. (In Press.)
71. Williams, R. J.: The Vitamin Requirement of Yeast. A Simple Biological Test for Vitamin. Jour. Biol. Chem. *38*, 465, 1919.
72. Bachmann, F. M.: Vitamin Requirements of Certain Yeasts. Jour. Biol. Chem. *29*, 235, 1919.
73. Schopfer, W. H., and Jung, A.: Un Test Végétal pour l'Aneurine: Méthode, Critique et Résultats. V° Cong. Int. Tech. Chim. d. Indust. Agric. Scheveningue 1937, p. 22.
74. Schultz, A., Atkin, L., and Frey, C. N.: Fermentation Test for Vitamin B_1. Jour. Amer. Chem. Soc. *59*, 948, 1937.
75. Williams, R. J.: The Use of Yeast or Other Fungi for Vitamin B_1 Tests. Science *86*, 348, 1937.
76. Williams, R. J., and Roehm, R. R.: The Effect of Antineuritic Vitamin Preparations on the Growth of Yeasts. Jour. Biol. Chem. *87*, 581, 1930.
77. Williams, R. J., and Saunders, D. H.: Effect of Inositol, Crystalline Vitamin B_1 and "Pantothenic Acid" on the Growth of Different Strains of Yeast. Jour. Biol. Chem. *28*, 1887, 1934.
78. Meiklejohn, A. P.: Estimation of Vitamin B_1 in Blood by a Modification of Schopfer's Test. Biochem. J. *31*, 1441, 1937.

CHAPTER XVIII

DISTRIBUTION OF THIAMIN IN FOODS

In considering from a quantitative standpoint the distribution of thiamin in foods, abundant data present themselves, but there are wide divergences among the assays of a particular foodstuff in many instances. This is so much the case that a reconciliation of the results of different workers is often impossible. To present all the existing data would merely burden and confuse the reader. Fortunately, however, the essential features of the picture can be outlined in a few pages. This will be done according to the authors' best judgment, leaving less essential details to be resolved and elaborated by future experimentation.

It is clear from large numbers of assays which have been made of a wide variety of animal and vegetable tissues that thiamin is of nearly universal occurrence in quantities usually ranging from 0.1 to 2.0 γ per gram. These proportions are exceeded in the plant world only in seeds and in yeast grown in rich media. Among the muscular tissues of animals, so far as is known, pork is outstanding. In other species the animal organs, liver, heart, and kidney, contain somewhat higher concentrations than the body at large. The task of estimating the thiamin supplied by mixed diets requires accurate estimates of the thiamin content of a very large number of foods, most of them containing amounts of thiamin below the range which present methods are ideally adapted to determine.

If one uses a rat growth method, the food under test displaces the basal diet very largely whenever the test food contains less than 1 γ per gram. Accordingly, many other factors besides thiamin content may influence the growth rate and disturb the measurement. Similar considerations apply in somewhat lesser degree to the more specific polyneuritis prevention and polyneuritis curative methods. For all of them a quantitative means of concentrating the thiamin content of foods is imperative if higher precision is to be obtained.

Little practical dietetic purpose is served by tabulating the thiamin content of minor foods in detail. We have, however, attempted to do so in the case of staples (see Tables X–XVI) to an extent sufficient to permit a rough appraisal of a variety of

diets which have been historically associated with outbreaks of human beriberi or with recessions of the disease. In doing so we have made liberal use of the data so painstakingly collected by Cowgill (1) and with the same object which he had in mind, namely, to arrive at a first approximation of the thiamin requirement of man. Incidentally, in so doing we subject our data on the thiamin content of foods to a test of their general validity by noting the precision of correspondence of the calculated thiamin content with the occurrence or non-occurrence of beriberi (see Chapter VII). In the tables which follow, some emphasis has been placed upon Oriental staples to facilitate the analysis of these diets.

In tabulating the thiamin content of foods, one is compelled to follow in certain particulars a somewhat less systematic procedure than he would like. Assay figures are often available for a particular food only in the raw state, although the food may appear in human diets customarily in the cooked condition. The process of preparing the food may affect its thiamin content in one or more of the following ways: (*a*) by destruction of thiamin by heat; (*b*) the leaching of thiamin into the cooking water, which may or may not be discarded; (*c*) bones, peelings, cores, seeds, etc., are often discarded in preparing food for the table, introducing questions as to the net weight of the food and entailing unknown losses of thiamin; (*d*) the water content and resultant final weight of the food may be greatly increased or reduced by cooking. We have included in the first four columns of the succeeding tables the observed thiamin content of the foodstuff as recorded in the literature, converting all values into the common term of γ per gram, which corresponds to parts per million. In the fifth column, under the heading "preferred value," we have endeavored to choose the most trustworthy value and to make suitable adjustment for the changes occurring in preparation of the food for consumption. In doing so, however, we have not included a correction factor for changes in water content during cooking because such changes depend greatly upon the character of the cooking process, and no particular cooking process can be regarded as standard. We have also had in mind that foods are more commonly weighed as purchased, not as ready for the table. The "preferred value" therefore aims to give one as definite an idea as possible of the amount of thiamin which one would actually realize from a unit quantity of the food as weighed in the raw state after it had undergone the customary preparation for human use. For example, if a housewife purchases one pound or one kilo of beef, she may learn from the "preferred value" column our best estimate of its thiamin "yield" after it has been cooked. We make no attempt to distinguish whether it has been stewed or broiled

or has gained or lost weight during cooking. In the case of foods eaten raw, the "raw" value of thiamin content appears in this column.

In thus stating explicitly our aim and method, we hope that we shall not mislead our readers by giving them an exaggerated notion of our pretensions as to the precision of our knowledge. In many instances the error of present-day assays is probably of a greater order of magnitude than the difference between the cooked and raw state of a given food. In general, however, we have felt we would not be justified in ignoring cooking losses altogether nor in attempting to make a difficult and somewhat arbitrary correction for gain or loss of water in cooking.

We will first discuss the data on animal tissues (Table X). This subject has been a matter of much dispute. Most of the assays of animal tissues which are available have been made by the rat growth and bradycardia methods, neither of which has been rigorously proved to be highly specific for the antineuritic factor or essentially free from complications due to other causes. Cooper (2) showed a number of years ago that animal foodstuffs do not rate high in polyneuritis-preventing properties. Hoagland confirmed this finding as to beef and found that the same was true of mutton. Pork, however, surprisingly enough, was found to be seven to eight times richer (3). Hoagland's method was the classical one of protection of pigeons against polyneuritis. He used the dried tissues to facilitate feeding of adequate quantities. This method often leads to lower estimates than the above-mentioned rat methods. In the case of fresh, undried foods of low thiamin content, failure to protect pigeons from polyneuritis is experienced even when the test food is given in amounts equal to or greater than the polished rice consumed. The literature reveals very few successes in preventing polyneuritis in pigeons with flesh foods, fruits, or green vegetables. The use of the rice bird in a similar way was also unsuccessful according to Van Veen (4) in the case of this class of foods, but on resorting to the rat growth method he was able to secure results reasonably in accord with those of other experimenters with rats. We view this circumstance as an indication that rat growth in the test in question is promoted in part by other substances than thiamin. Lacking bird values for flesh foods, we have substituted determinations by the chick method of Elvehjem in column 4. A series of experiments with pigeons in which large amounts of animal foodstuffs were found to be necessary to protect against polyneuritis was that of Plimmer *et al.* (5). We have not used their values, as their test period was of twenty-six weeks' duration, a length of time exceeding that which has been used in such experiments with pure thiamin. Accordingly, we lack means of converting their values to quantities of thiamin.

Dairy products (Table XI) represent a less troublesome field, as there is general agreement that butter and cheese furnish negligible amounts of thiamin; that cow's milk and eggs are fairly rich, though not equal to cereals in thiamin content per unit of dry weight. Of course, milk is much inferior to cereals weight for weight in the natural state.

The legumes (Table XII) constitute a fairly important source of thiamin which is, however, substantially diminishd by loss in cooking waters and in certain instances by heat destruction during the long process of cooking which is needed to render the ripe seeds digestible.

The cereals (Table XIII) rank first, as all authorities agree, as the most important potential source of thiamin in human diets. The high starch content, relatively low water content of cereals, as well as their cheapness, are responsible for the fact that they represent one of the chief reliances of the race for its supply of calories. The high starch content of seeds, in turn, makes their high thiamin content necessary to their proper utilization as food because of the special relationship of thiamin to carbohydrate metabolism. The high thiamin content also appears to be significant with respect to plant function in view of the evidence of need for it during germination of the seed (see Chapter XXVIII).

Fortunately the various methods of assay apparently are better adapted to the study of seeds than to many other foodstuffs, for the discrepancies are fewer and less serious. In spite of the great importance of cereals, very few of them have been subjected to elaborate studies of thiamin content. Happily, this has been done for rice and to a lesser extent for wheat, the two great staple grains of the world. J. P. Spruyt (see footnotes of Table XIII) assayed 184 kinds of rice by two different chemical tests, and in 126 of these cases secured bioassays with rice birds for comparison with the chemical tests. In these bioassays 5550 birds were used. European and especially British workers have recently been addressing themselves quite earnestly to detailed studies of wheat.

Spruyt's results indicate no wide gulf between the white, more or less polished rices and those which are unpolished. (He does not give detailed descriptions of all the rices examined.) The thiamin content grows progressively larger with less milling, but there is no sharp dividing line among the raw rices. As prepared for human consumption, the line of division becomes much sharper as the polished rices lose a larger proportion of their thiamin in the process of washing preparatory to cooking. However, Spruyt applied in all cases an iodine test to the grains which permitted him to grade them by numbers ranging from 1 to 9 with respect to the extent of the exposure of the starchy part

of the grain. The data are given below for most of those rices for which some specific description of the degree of decortication was furnished.

No.	Description	Iodine Test	Bird Test, γ per Gram
1	Polished and washed	9	.02
47	Unmilled	2	2.40
48	Unmilled	2	3.00
49	Milled	7	1.00
50	Milled	7	1.20
51	Milled	7	.80
52	Milled	8	.64
53	Milled	7	.50
54	"Table rice"	8	.30
62	Half milled	5	1.24
63	Well milled	7	.56
72	Unmilled	4	2.16
73	Twice milled	8–9	.34
74	Thrice milled	9	.60
75	Well milled	9	.58
89	Well milled red rice	9	.60
142	Milled	9	.60
172	Hand pounded	4–5	1.72
173	Hand pounded	4–5	1.20
180	Hand pounded	1–2	2.70
183	Unmilled	—	2.60
184	Unmilled	—	2.72

For the purpose of the animal assays, Spruyt made extracts of the rices and evaporated these extracts upon known quantities of previously well-washed polished rice. It is a type of method which should be more widely emulated.

We have considered it reasonable to suppose, from personal observation of the habits of Oriental peoples, that rice is prevailingly subjected to washing before cooking. As rice is prevailingly eaten with the fingers, it is a matter of pride to avoid stickiness of the grains. This is facilitated by washing off the loose starch. In view of the experience of the Dutch workers that washing reduces the thiamin content of polished rice quite markedly (6), a value of 0.3 γ per gram of washed grain seems reasonable as an average figure. This coincides with the vitamin index used by Cowgill for polished rice. It should be remembered that there is no such commercial article as whole rice available as human food. The removal of the outer palets of the whole grain or paddy inevitably removes some of the bran also, as the palets are closely adherent to the seed. An approximation to whole rice is parboiled rice. In this case the loosening of the palets is effected by a light steaming. Aykroyd, who has studied this process, reports that it results in disseminating the vitamin through the endosperm so that subsequent decortication does

not deprive it of its thiamin to nearly so large an extent as is the case with unparboiled rice. This process is popular as a household measure in some localities but is not generally used throughout the Orient. Presumably modernization of the East has tended to diminish the practice.

The milling practices vary from country to country and from one mill to another. So do the terms rice bran, rice meal, rice polish, etc. In commercial utilization of these products for thiamin production, the greatest difficulty was encountered in securing even approximate constancy of quality from shipment to shipment, even when dealing with a single mill. The terms, therefore, have no very definite meaning for our purposes. All represent the products of successive abrasions of the exterior of the grain which are "cut" into various "streams" according to the judgment of the operator.

There are varieties of rice, both in the Orient and in America, which are referred to as red rice, as the exterior of the endosperm is of a distinct reddish color. These rices do not differ in thiamin content from the ordinary white rices in equal state of milling. The term brown rice means rice which retains some of the bran. Parboiled rice is brown rice, but the parboiling process reddens the grains somewhat and this fact has given rise to some confusion of the terms brown and red.

As is the case for rice, the milling processes used in preparing wheat vary from country to country, from mill to mill, and from variety to variety of wheat so that the white flours grade into the "clear" flours and these into the graham, whole meal, or whole wheat flours. The decortication of wheat in the process of milling, of course, depends on the toughness of the bran coats and the coherence of the germ, which tend to keep those parts in a coarse state so that they remain on the sieves while the more starchy part passes through. Some of the recent assays of various flours and breads are given below, translated into γ per gram: Results of Copping and Roscoe:

"A" Blend of Wheat	*γ per Gram*
Whole meal flour	4.72
Straight-run flour (70–72 per cent of wheat)	1.48
Straight-run flour bleached with NO_2	1.48

The thiamin content of these straight-run flours did not change during nine to twelve months of storage.

"B" Blend of Wheat	*γ per Gram*
Straight-run flour (70–72 per cent)	1.48
Patent (42 per cent)	0

The above results are by rat growth tests (7).

Baker and Wright (8) by the bradycardia test recorded values for three whole wheats corresponding to 6.9, 7.8, and 10.2 γ per

gram. Scheunert and Schieblich (9), by a little-used rat protective method, found 2.4, 3.0, and 3.0 γ per gram for three whole wheats. Our method of converting their values to γ thiamin is, however, somewhat uncertain because of limited experience with the low levels of maintenance which they used. Their standard was the amount of the cereal product required to keep young rats alive without loss of more than 2 grams in weight during thirty-five days and without symptoms of polyneuritis. This unit is obviously less than a Chase-Sherman Unit, which permits 3 grams' growth per week. From Ammerman and Waterman's results, page 204, we estimate the pure thiamin required to do this at about 1.2 γ daily. Since Scheunert and Schieblich found that 6 milligrams of International Standard clay daily would just meet their criterion, the conversion factor we have used in this case corresponds to 1 I.U. = 2γ. This is a very popular conversion factor with German writers (10).

Baker, Wright, and Drummond (11) by the bradycardia method secured figures corresponding to 3.6, 6.0, 6.3, 6.9, 7.5, and 7.8 γ per gram for six wheats; Leong and Harris (14), 4.5 γ per gram for one.

In flour of 60 per cent extraction, Scheunert (12) found no thiamin; flour of 75 per cent extraction retained 40 per cent of that of the whole grain; of 82 per cent extraction, 60 per cent of that of the whole grain. The method was again the rat protective one. Scheunert and Schieblich (13), by the same method, recorded 3.0 γ per gram for whole wheat; 2.4 γ for 94 per cent; 2.0 γ for 82 per cent; 0.8 γ for 75 per cent; 0.48 γ for 60 per cent. Leong and Harris (14) reported 0.9 γ and 0.6 γ in two white flours by bradycardia test. The present author many years ago found that polyneuritis developed very tardily in pigeons on a 71 per cent flour, very promptly on a 49 per cent flour from the same wheat, and not at all on a 79 per cent flour when these were used as exclusive diets. This should correspond to about 0.6 γ per gram for the 71 per cent flour. From all of the above results we feel we are quite justified in regarding the bradycardia results as often too high.

In yeast breads there appears to be fairly general agreement that the thiamin content on an air-dry basis corresponds very closely to that of the flours used. The yeast which is used in proportions of 1 part to 100 of flour or less does not add significantly to the thiamin content. Copping and Roscoe's results by the rat growth method are typical:

	Air Dried	*Fresh*	
White, sponge	1.0	0.68	from 72 per cent flours
White, short dough	1.48	1.0	
White, long dough	1.48	1.0	
Whole meal	4.72	3.0	

In baking powder bread a substantial fraction of the thiamin was found to be destroyed. This can be corrected by rendering the dough acid (15).

In transferring our attention to vegetables (Table XIV) and fruits, we again enter an area of dispute and uncertainty. The high water content of these food articles increases very greatly the difficulties of properly assaying them in the fresh state. In general it is not possible to protect pigeons or rice birds from polyneuritis by feeding fresh fruits and vegetables in admixture with polished rice. In consequence, many of these foods are reported in the Oriental literature as substantially free from vitamin B_1 (16, 17). On the other hand, the rat workers who have frequently dealt with the dry substances have in general reported appreciable values. Inasmuch as fruits and vegetables contain from 75 to 96 per cent water (usually 90 per cent or more) in the fresh state, and as the water content of the dried product is often not stated, a substantial uncertainty is involved. Obviously the water content of the "fresh" or "dried" product is likely to vary during the course of a long feeding experiment due to exposure to the air. Further, for some reason the bradycardia method seems to give higher values than any other, particularly for this class of foods. In choosing preferred values for the table on thiamin content of fresh vegetables, we have leaned toward lower estimates because such assays as we have been able to make by the rat curative method of extracts of such foodstuffs seem to justify a skepticism of the higher values. Further, the practical consideration that vegetables under prevailing cooking practices are peculiarly subject to losses of thiamin in cooking waters points in the same direction.

Whatever the actual thiamin content of leaves, roots, and stems, their high water content and great bulk prevent them from playing a very important role as thiamin sources in human dietaries. One cannot eat large amounts of solids in such forms. Desiccated leaves, roots, and fruits, however, often approach whole seeds in thiamin content.

Attention should be called at this point to the evidence that the thiamin contents of green plants decline with advancing age and are at a maximum in the young tender growth (18). Variability in age or state of advancement may be an important cause of apparently conflicting data in the literature on the thiamin content of leafy vegetables.

The association of seeds with fruits suggests the possibility of some concentration of thiamin in the fruit (Table XV). The fruit pulp is the medium in which the maturation of the seed occurs, and it might well reflect the progress of the process of storage of thiamin which takes place in the seed. That this feature is not more conspicuous than it appears may well be due

to the fact that the pulpy portions of most fruits used as food have been grossly overdeveloped by processes of artificial selection. These heavy pulps external to the seed serve no plant function other than to facilitate scattering of the seeds by making the fruits attractive to animals. Nuts seem to reveal in general the high thiamin contents characteristic of seeds; namely about 4–5 γ per gram on the average. Fruit pulps, like vegetables, are so high in water content that the contribution to the solids of dietaries is quite low. Fruits, however, are less subject to cooking losses than vegetables because their prevailingly high acidity protects the thiamin from destruction; the cooking waters are infrequently discarded, but rather serve, usually with the added sugar, as the most toothsome part of the dish. In addition, fruits are much more frequently eaten raw than are vegetables. In spite of these facts, the fruits do not constitute a very important source of vitamin B_1. Their content of vitamin C is, of course, highly significant.

In the miscellaneous category of foods (Table XVI) sugar is the most important item, as in the Western world, and especially in America, it furnishes a large caloric supply, particularly to children. Refined sugar is devoid of thiamin. Regarding molasses there is a serious question. Nelson, Heller, and Fulmer (19), reported a high antineuritic value as judged by rat growth tests. The present authors seized upon this as a possible convenient source of the vitamin for purposes of large-scale isolation of the vitamin, as waste cane molasses is brought into the United States in tank steamers for use by the fermentation and yeast industries. A study of the matter revealed only negligible amounts of antineuritic vitamin in such molasses. This form of molasses did not protect pigeons when given in amounts up to 5 grams on alternate days, in spite of the fact that the nitrogenous bases present should have been highly concentrated by extraction of the sucrose and subsequent further concentration for removal of all possible sugar.

We noted a great contrast in Cowgill's values for sugar-cane juice (0) and for molasses (150 mg. equiv.) (1). Molasses apparently had made an important contribution to the Newfoundland diets reported by Aykroyd and discussed by Cowgill. Accordingly we assayed five samples of clear commercial cane sirup * by the rat curative method (page 207) and found that more than 50 grams were required to yield enough vitamin to cure a rat (5γ). This low content is in agreement with another study, which resulted in the conclusion that molasses is "devoid of demonstrable quantities of vitamin B" (20). Yet cane juice, according to this study, contains appreciable amounts, although

* One much richer sample has recently been encountered. The use of alkali or sulfur dioxide in manufacture may be significant.

not enough to account for more than 2.0 γ per gram in molasses, assuming the latter represents a fivefold concentration (75 per cent sucrose).

Other miscellaneous foodstuffs of some importance are tapioca, sago, macaroni, and spaghetti, all of which contain negligible amounts of thiamin. The beverages coffee, tea, and beer (21) furnish no significant supply. Chocolate is said to contain an amount equivalent to about one part per million (22), and since it is extensively used in confections, it may have a slight significance.

There are, of course, large numbers of other plant and animal tissues used as food besides those which are listed in the above tables. A more complete presentation seems undesirable, not only because of unnecessary consumption of space but also because of great uncertainty of the true values. Many of the observed values are set down, in spite of the fact that we have little faith in them, merely to illustrate the existing discrepancies. Those given in the preferred-value column are believed to be representative for their respective classes and are adequate to support the thesis that while thiamin is well-nigh omnipresent in foods, it is nowhere very abundant. The seeds, milk, and tissues of the swine stand out as the principal sources of enrichment of the diet with respect to this factor.

Although yeast can scarcely be said to be a regular article of human food, its thiamin content is of general interest in this connection. Quinn, Whalen, and Hartley (23) found by the rat growth test a tenfold variation from bakers' to brewers' yeasts. This has been amply borne out by other workers. Copping and Roscoe (7) found for a brewers' yeast about 140 γ per gram and for a special bakers' yeast 80 γ. For two ordinary bakers' yeasts 28 γ and 40 γ respectively were found. There has been a marked tendency in recent years to build up the thiamin content of bakers' yeasts by improving the media on which they are grown. Pavcek, Peterson, and Elvehjem (24) report the thiamin contents of various yeasts as determined by the chick method of assay.

Medium on Which Grown	*γ per Gram (Dry)*
Glucose	12
Molasses	20
Grain	40

Commercial yeasts were found to vary from 15 γ to 42 γ for bakers' and 42 γ to 160 γ for brewers'. Similar high values for thirty brewers' yeasts are reported by Scheunert (25). The range corresponded to 48 γ to 360 γ per gram of dry yeast. The higher values are associated with rich media in which are grown relatively small crops of yeast. If supplied with traces of thiamin,

yeast appears capable of synthesizing more but it supplements synthesis extensively by capture from the medium.

For those who wish confirmatory or supplementary information, we wish to call attention to an excellent and rather conservative summary of the vitamin B_1 contents of foods which has appeared recently (26). It may be obtained from the Superintendent of Documents, Washington, D.C., for 15 cents. Attention is called to conflicting statements about the significance of the star (*) in Tables 1 and 3 of this publication. Evidently that at the foot of the latter table is correct. The data of Table 3 are preferable as they represent a critical selection and are uniformly in Sherman units.

TABLE X

THIAMIN CONTENT OF ANIMAL TISSUES

(γ per gram or parts per million)

	RAT GROWTH	BRADYCARDIA	PIGEON PROTECTIVE	CHICK TEST [4]	PREFERRED VALUE	REMARKS
Beef musele, raw	0.9 [1]	1.5 [3]	0.25 [2]	—	0.5	
Beef musele, raw	1.7 [6]	—	—	—	—	
Beef muscle, cooked	0.7 [1]	—	—	—	0.4	
Beef muscle, dried	3.7 [1]	—	0.5 [2]	2.0	1.0	
Beef heart, dried	—	—	—	9.0	—	
Beef kidney, dried	—	—	—	14.0	—	
Beef liver, raw	3.0 [6]	—	—	—	—	See footnote 4.
Beef liver, cooked	—	4.5 [3]	—	—	2.0	
Veal, cooked	—	1.5 [3]	—	—	0.4	
Chicken muscle, roast	—	1.2 [3]	—	—	0.4 [7]	
Lamb muscle, raw	1.5 [5]	—	—	—	—	
Lamb muscle, roast	—	1.5 [3]	—	—	0.5	
Lamb liver	3.0 [5]	—	—	—	—	
Lamb heart	3.0 [6]	—	—	—	—	
Mutton, raw	—	1.8 [3]	—	—	—	
Mutton, cooked	—	—	—	—	0.5	
Mutton, dried	—	—	—	2.0	—	
Pork musele, fresh	10.5 [1]	—	2.0 [2]	—	—	
Pork musele, cooked	9.0 [1]	9.6 [3]	2.0 [2]	—	3.0	
Pork musele, dried raw	36.0 [1]	—	4.0 [2]	23.0	—	
Pork, boiled smoked ham	—	6.6 [3]	—	—	—	
Pork heart, dried	—	—	—	9.0	—	
Pork kidney, raw	—	10.2 [3]	—	—	—	
Pork kidney, dried	—	—	—	14.0	—	
Bacon	—	—	—	—	1.0	
Fish						
Raw eod muscle	—	1.2 [3]	—	—	—	
Raw whiting	—	0.9 [3]	—	—	—	
Fried halibut	—	1.8 [3]	—	—	—	
Tinned sardine	—	0.9 [3]	—	—	—	
Shrimps	—	0.9 [3]	0 [5]	—	—	
Fresh unspeeified	—	—	0 [5]	—	0.4	
Dried unspeeified	—	—	< 2.0 [8]	—	1.0	

[1] Christensen *et al.*, Jour. Agri. Res. *53*, 415 (1936).
[2] Hoagland, Jour. Agri. Res. *38*, 431 (1929).
[3] Baker and Wright, Biochem. Jour. *29*, 1802 (1935).
[4] The figures in this column are from unpublished data very kindly supplied by Dr. C. A. Elvehjem, and are based on the calculation that 70 γ of thiamin per 100 grams of ration are required to protect chicks as reported by Elvehjem and associates at American Chemical Society Meeting, Rochester, September 1937. 2 per cent whole dried beef liver "supplying a trace of B_1" was contained in the basal ration. High losses were noted in canning of pork.
[5] Pierson, S. Dak. Agr. Exp. Sta. Ann. Rept. 1934, p. 34.
[6] Roscoe, Biochem. Jour. *25*, 2050 (1931).
[7] Cf. Kemmerer *et al.*, J. Biol. Chem. *103*, 353 (1933).
[8] Jansen, Mededeel. Burg. Geneesk. Dienst. *1*, 1 (1923).

TABLE XI

Thiamin Content of Dairy Products

(γ per gram or parts per million)

	Rat Growth	Brady-cardia	Pigeon Protective	Rice Bird	Preferred Value
Milk, whole	0.48 [3]	0.7 [1]	—	0.45 [4]	0.45
Milk powder	—	—	1.6 [2,4]	—	2.5
Cheese	—	0.5 [1]	—	—	0.5
Egg, hen's whole	0.8 [3]	1.4 [1]	—	—	1.0
Egg, hen's whole	1.7 [5]	—	—	—	—
Egg, hen's white	0	—	—	—	0
Egg, hen's yolk [6]	5.0 [5]	—	—	—	3.0
Egg, duck	—	—	4.0 [2]	—	4.0

[1] Baker and Wright, Biochem. Jour. *29*. 1802 (1935).
[2] Jansen, Mededeel. Burg. Geneesk. Dienst. Ned. Ind. *1*, 1–118 (1923).
Donath, Mededeel. Dienst. Volksgezond. Ned. Ind. *18*, 334 (1929).
[3] M. S. Rose, Laboratory Handbook for Dietetics, Table XVIII.
[4] Spruyt and Donath, Mededeel. Dienst. Volksgezond. Ned. Ind. *21*, 64 (1932).
[5] Roscoe, Biochem. Jour. *25*, 2050 (1931).
[6] An egg is one third yolk approximately.

TABLE XII

Thiamin Content of Legumes

(γ per gram or parts per million)

Legumes	Rat Growth	Brady-cardia	Pigeon Protec-tive	Rice Bird	Pre-ferred Value	Remarks
Beans, soy, raw	—	—	2.0 [2]	3.5 [1]	3.0	
Beans, mongo	—	—	2.8 [2]	5.0 [1]	4.0	
Beans, haricot, dry	—	3.6 [3]	—	—	—	
Beans, butter, dry	—	4.8 [3]	—	—	—	
Beans, baked, canned	—	Trace [3]	—	—	—	
Beans, kidney	—	—	3.0 [2]	—	—	
Beans, green, unspecified	0.25 [5]	—	—	—	1.0	
Beans, French	—	—	< 1.6 [2]	—	—	
Beans, pinto, raw	15.0 [4]	—	—	—	—	
Beans, string	0.48 [7]	—	—	—	0.5	
Beans, unspecified	—	—	—	—	2.5	
Dhal or Dal	—	—	—	—	3.0	
Dhal, sprouted	—	—	—	—	2.0	
Lentils	—	6.3 [3]	—	—	—	
Peas, canned	0.4 [5]	3.6 [3]	—	—	1.0	
Peas, dried green	—	—	—	4.0 [1]	3.0	
Peas, green	3.1 [7]	—	—	—	1.5	
Peanuts	—	—	—	8.0 [1]	8.0	

A wide variety of beans or lentils are covered by this Indian term.[9] Often they are eaten in sprouted form in India [6] as well as in China [10] and vary over a wide range in B_1 content.

[1] Van Veen, Geneesk. Tijdschr. Ned. Ind. *75*, 2050 (1935).
[2] Jansen, Mededeel. Burg. Geneesk. Dienst. *1*, 1 (1923)
Donath, Mededeel. Dienst. Volksgesond. Ned. Ind. *18*, 334 (1929).
[3] Baker and Wright, Biochem. Jour. *29*, 1802 (1935).
[4] Greenwood, M. L., N. Mex. Agr. Exp. Sta. Bull. *232*, 3 (1935).
[5] Hanning, J. Nutrition *8*, 449 (1934), refers to strained vegetables.
[6] Hebir, P., Indian Jour. Med. Res. *7*, Spec. No. Ind. Sci. Cong. 57 (1919).
[7] M. S. Rose, Laboratory Handbook for Dietetics, Table XVIII.
[8] H. E. Sherman, Philip. Jour. Sci. *38*, 1 (1929).
[9] Ghose, Biochem. Jour. *16*, 35 (1922).
[10] H. E. Sherman, Philip. Jour. Sci. *38*, 9 (1929); J. Hlavaty, Compt. Rend. Soc. Biol. *100*, 587 (1929); E. H. Funnell, Dissertation, Columbia University (1935).

TABLE XIII

Thiamin Content of Cereals

(γ per gram or parts of million)

Cereals	Rat Growth	Brady-cardia	Pigeon Protective	Rice Bird	Preferred Value	Remarks
Attah	—	—	—	—	4.0	A mixture of various flours, *e.g.*, wheat, pea, and maize, retaining the outer coats.[11]
Barley, whole	—	—	—	—	4.0	
Barley, pearled	—	—	—	—	0.5	
Barley, germ	—	42 [2]	—	—	—	
Buckwheat	—	—	2.0 [12]	—	—	
Maize, ripe	—	—	1	2–3 [2]	2.5	
Maize, unripe	—	—	1	—	2.0	
Maize, meal	—	—	< 0.7	—	0.5	
Maize, germ	—	14	—	—	—	
Millet	—	—	2.0 [1]	—	2.0	
Oatmeal	—	4–10	—	—	4.0	
Oats, rolled	3.3 [9]	—	—	—	3.0	
Rice, polished	—	—	—	0.3–1.0 [4]	0.5	
Rice, polished, washed	—	—	—	0.02 [4]	Trace	
Rice, red — same as white	—	—	—	—	—	
Rice, unpolished	—	—	—	1.0–3.0 [4]	2.2	
Rice, cured [8]	—	—	—	—	2.5	
Rice, polish	20 to 37.5 γ by rat curative tests of J. C. Keresztesy and W. L. Sampson					
Rice, bran	—	17–23 Same as rice polish by rat curative tests.				
Rice, white in human diets	—	—	—	—	0.3	
Rye, grain	—	—	2.0 [10]	—	2.0	The values in third column for rye products were obtained by a special rat protective method.[10]
Rye, flour	—	—	1.7 [10]	—	1.7	
Rye, germ	—	22 [2]	6.0 [10]	—	—	
Sorghums	4.0 [5]	—	—	—	—	
Wheat, whole berry	—	7–10 [2]	—	—	5.0	5 γ per gm. by rat curative test
Wheat, flour, graham	3.3 [9]	—	—	—	3.3	
Wheat, flour, straight run	—	—	—	—	1.0	
Wheat, flour, patent	—	0.45 [7]	—	—	0.5	
Wheat, flour, whole	—	4.5 [7]	—	—	4.0	
Wheat, bread, whole	4–5.2 [6]	3.6 [7]	—	—	4.0	
Wheat, bread, white	1.2 [6]	0.4–0.6 [7]	0.6 [1]	—	0.5	
Wheat, bread, off white	—	—	—	—	1.0	
Wheat, germ	—	18–56 [2]	—	—	40.0	Varies with milling process.
Wheat, bran	—	4–9 [2]	—	—	—	

[1] Jansen, B. C. P., Mededeel. Burg. Geneesk. Dienst. Ned. Ind. *1*. 1 (1923).
[2] Baker and Wright, Biochem. Jour. *29*, 1802 (1935).
[3] Van Veen, Geneesk. Tijdschr. Ned. Ind. *75*, 2050 (1935).
[4] Spruyt, J. P., Doctor's Thesis, University of Amsterdam, 1933.
[5] Reder, R., Proc. Okla. Acad. Sci. *14*, 50 (1934).
[6] Morgan and Frederick, Cereal Chem. *12*, 390 (1935).
[7] Baker, Wright, and Drummond, Chem. & Ind. *56*, 292, 191T (1937). See also Harris, L. J., Biochem. Jour. *31*, 799 (1937) and Leong, P. C. and Harris, L. J., Biochem. Jour. *31*, 812 (1937).
[8] Aykroyd, W. R., Jour. Hygiene *32*, 184 (1932).
[9] Rose, M. S., Laboratory Handbook for Dietetics, Table XVIII.
[10] Scheunert, A., and Schieblich, M., Biochem. Zeit. *290*, 398 (1937).
[11] Ghose, Biochem. Jour. *16*, 35 (1922).
[12] Yarusova, U. S., Z. Vitaminforsch. *6*, 98 (1937).

TABLE XIV

Thiamin Content of Fresh Vegetables

(γ per gram or parts per million)

	Rat Growth	Brady-cardia	Pigeon Protective	Rice Birds	Preferred Value	Remarks
Beets	—	2.1 [1]	—	—	0.5	
Cabbage, etiolated	1.0 [2]	—	—	—	—	
Cabbage, green	0.4 [2], 0.42 [4]	2.0 [1]	0 [5]	—	0.2	0.12 γ per gram by rat curative tests in cooked cabbage
Carrots, raw	2.0 [2], 0.4 [4]	1.8 [1]	—	—	0.4	
Cauliflower	—	0.9–3.3 [1]	—	—	0.2	
Celery	—	Trace [1]	—	—	—	
Cucumber	—	0.9 [1]	0 [5]	—	—	
Eggplant	—	—	—	0.3 [3]	—	
Greens, unspecified	—	—	—	—	0.5	
Lettuce	0.4 [2], 0.58 [4]	2.7 [1]	—	—	0.2	0.18 γ per gram by rat curative tests
Onion	0.4 [2], 0.3 [4]	1.2 [1]	—	—	0.2	
Potato	0.75 [2], 0.42 [4]	0.9–1.2 [1]	1.3 [5]	—	0.7	0.67 γ per gram in boiled potato by rat curative tests
Radish	0.5	1.8 [1]	—	—	0.4	
Spinach	0.8 [2], 1.16 [4]	2.1 [1]	< 1.0 [5]	< 1.0 [3]	0.5	5 gm. dried spinach daily failed to protect pigeons in authors' experiments
Sweet potato	—	—	1.3 [5]	—	0.7	
Turnip	0.4 [4], 0.5 [2]	Trace [1]	—	—	0.5	
Turnip greens	1.8 [4]	—	—	—	—	
Rutabagas	0.4 [4]	—	—	—	—	
Vegetables unspecified	—	—	—	—	0.5	
Watercress	0.7 [2]	1.8 [1]	—	—	0.5	

[1] Baker and Wright, Biochem. Jour. *29*, 1802 (1935).
[2] Roscoe, M. H., Biochem. Jour. *24*, 1754 (1930), *25*, 1205 (1931).
[3] Van Veen, A. G., Geneesk. Tijdschr. Ned. Ind. *75*, 2050 (1935).
[4] Rose, M. S., Laboratory Handbook for Dietetics, Table XVIII.
[5] Jansen, B. C. P., Mededeel. Burg. Geneesk. Dienst. Ned. Ind. *1*, 1 (1923).

TABLE XV

Thiamin Content of Fruits

(γ per gram or parts per million)

Fruits	Rat Growth	Brady-cardia	Pigeon Protective	Rice Bird	Preferred Value	Remarks
Apple	0.2 [1]; 0.4 [7]	1.2 [2]	—	—	0.2	
Banana	0.4 [1]; 0.4 [7]	1.5 [2]	0.4 [6]	—	0.4	
Fig, dried	0.7 [6]	3.0 [2]	—	—	—	
Grape	—	Trace	—	—	—	
Grapefruit	—	1.2 [2]	—	—	—	
Orange	0.25 [1]; 0.48 [7]	1.2 [2]	—	—	0.6	0.6 γ per gram by rat curative tests
Peach	0.42 [7]	—	—	—	—	
Pear	—	0.9 [2]	—	—	—	
Pineapple juice	1.0 [4]	—	—	—	—	
Plum	—	2.7 [2]	—	—	—	
Prune, dried	2.0 [7]	2.2 [7]	—	—	2.0	
Tomato	1.2 [5]; 0.6 [7]; 0.2 [1]	—	—	—	0.5	0.5 γ per gram by rat curative tests
Fruits, unspecified fresh	—	—	—	—	0.5	
Fruits, unspecified dried	—	—	—	—	2.0	

[1] Roscoe, M. H., Biochem. Jour. *25*, 2050 (1931).
[2] Baker and Wright, Biochem. Jour. *29*, 1802 (1935).
[3] Jansen, B. C. P., Mededeel. Burg. Geneesk. Dienst. *1*, 1 (1923). No protection with banana as sole food.
[4] Guerrant, Dutcher, *et al.*, Jour. Nutrition *11*, 383 (1936).
[5] Morgan, Hunt, and Squier, Jour. Nutrition *9*, 395 (1935).
[6] Morgan, Field, *et al.*, Jour. Nutrition *9*, 383 (1935).
[7] Rose, M. S., Laboratory Handbook for Dietetics, Table XVIII.

TABLE XVI

Thiamin Content of Miscellaneous Foods

	Preferred Values per Gram	
Beverages		
Beer	Trace	
Chocolate	1.0?	
Coffee	Trace	
Tea	Trace	
Fats		
Lard	0	see page 266
Salad oils	0	see page 266
Vegetable frying fats	0	see page 266
Nuts		
Almond	1.5	
Chestnut	1.7	
Hazel nut	3.3	
Pecan	1.5	
Starchy Foods		
Tapioca	0.1	
Sago	0.1	
Macaroni	0.5	
Spaghetti	0.5	
Sweets		
Honey	0	
Sugar	0	
Molasses	0.1	see page 234
Yeast, bakers'	20	see page 235
Yeast, brewers'	70	see page 235

BIBLIOGRAPHY

1. Cowgill, G. R.: Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
2. Cooper, E. A.: On the Protective and Curative Properties of Certain Foodstuffs against Polyneuritis Induced in Birds by a Diet of Polished Rice. Jour. Hgy. *14*, 12, 1914.
3. Hoagland, R.: Antineuritic and Water-soluble "B" Vitamins in Beef and Pork. Jour. Agric. Res. *38*, 431, 1929.
4. Van Veen, A. G.: Het B_1 gehalte der voornaamste Indische voedingsmiddelen. Geneesk. Tijdschr. Ned. Ind. *75*, 2050, 1935.
5. Plimmer, R. H. A., Raymond, W. H., and Lowndes, J.: Comparative Vitamin B_1 Values of Animal Foodstuffs. Biochem. Jour. *27*, 58, 1933.
6. Van Veen, A. G.: The Effect of Washing and Steaming Rice of Different Polishing Degrees on Its Content of B_1 Vitamin. Geneesk. Tijdschr. Ned. Ind. *73*, 945, 1933.
7. Copping, A. M., and Roscoe, M. H.: Water Soluble B Vitamins in Yeast, Flour and Bread. Biochem. J. *31*, 1879, 1937.
8. Baker, A. Z., and Wright, M. D.: The Vitamin B_1 Content of Foods. Biochem. J. *29*, 1802, 1935.
9. Scheunert, A., and Schieblich, M.: Über den Einfluss der Düngung auf den Vitamin-B_1-Gehalt von Weizen. Biedermann's Zentr. B. Tierernährung *8*, 120, 1936.
10. Stepp, W., Kühnau, J., and Schroeder, H.: Die Vitamine und ihre klinische Anwendung, p. 39. Stuttgart, Ferdinand Enke, 1937.
11. Baker, A. Z., Wright, M. D., and Drummond, J. C.: Nutritive Value of Bread. Jour. Soc. Chem. Ind. *56*, 191T, 1937.
12. Scheunert, M.: Zur Frage der Bedeutung von Mehl und Brot für die Deckung des Vitamin-B-Bedarfes. Ernährung *1*, 53, 1936.
13. Scheunert, A., and Schieblich, M.: Über den Vitamingehalt von Weisen und Roggen und der daraus hergestellten Mehle und Brote. Biochem. Zeit. *290*, 398, 1937.
14. Leong, P. C., and Harris, L. J. CVII. Vitamins in Human Nutrition. Vitamin B_1 and the "Brown versus White Bread Problem." Biochem. J. *31*, 812, 1937.
15. Khlestkina, Z. N.: Enrichment of Biscuits with Vitamin B_1. Proc. Sci. Inst. Vitamin Research U.S.S.R. *2*, 29, 1937.
16. Donath, W. F.: Synopsis of Anti-xerophthalmic, Antineuritic and Antiscorbutic Vitamin Percentages . . . of Different Indian Foodstuffs. Mededeel. Dienst. Volksgezond. Ned. Ind. *18*, 334, 1929.
17. Donath, W. F., Koolhaas, D. R., and Van Veen, A. G.: Tables Showing Chemical Composition of Native Food Products in Netherlands East Indies. Geneesk. Tijdschr. Ned. Ind. *75*, 426, 1935.

18. Hunt, C. H., Record, P. R., and Bethke, R. M.: Effect of Stage of Maturity and Method of Curing upon the Vitamin B and Vitamin G Content of Alfalfa, Clover and Timothy Hays. Jour. Agric. Res. *51*, 251, 1935.
19. Nelson, V. E., Heller, V. G., and Fulmer, E. I.: Molasses as a Source of Vitamin B. Ind. and Eng. Chem. *17*, 199, 1925.
20. Nelson, E. M., and Jones, B.: Vitamins in Sugarcane Juice and in Some Canejuice Products. Jour. Agric. Res. *41*, 749, 1930.
21. Scheunert, A., and Schieblich, M.: Über den Vitamingehalt des Bieres. Zeit. f. Vitaminforsch. *4*, 294, 1935.
22. Sasaki, R., and Wahoyama, H.: Vitamin B in Chocolate. Jour. Agric. Chem. Soc. Japan *8*, 160, 1932.
23. Quinn, E. J., Whalen, F. B., and Hartley, J. G.: Vitamin B and G Contents of Certain Yeast Samples. Jour. Nutrition *3*, 257, 1930.
24. Pavcek, P. L., Peterson, W. H., and Elvehjem, C. A.: Effect of Growth Conditions on Yield and Vitamin B_1 of Yeast. Ind. Eng. Chem. *29*, 536, 1937.
25. Scheunert, A.: The Vitamin B_1 and B_2 Contents of Fresh Yeast, Dried Yeast and Yeast Extracts. IV. Cong. intern. tech. chim. Agr. Bruxelles *2*, 52, 1935.
26. Daniel, E. P., and Munsell, H. E.: Vitamin Content of Foods. U. S. Dept. Agric. Misc. Pub. No. 275, 1937.

CHAPTER XIX

STORAGE AND EXCRETION

The tissues of experimental animals contain thiamin in greater or lesser amounts, depending upon the thiamin content of the diets to which the animals have been subjected. The excreta also regularly contain thiamin in greater or lesser amount, depending on the current intake of the substance in the diet. However, the proportion of thiamin stored to that excreted or destroyed by metabolic processes is not yet well established for many species and probably depends on many as yet ill-defined factors. It has, however, become clear that the body is not capable of storing large supplies of thiamin against a future time of need but tends to waste what it cannot immediately use. In general, it holds in reserve at a given time only a sufficient supply to care for its absolute necessities during a period of a few weeks. The body is not, however, an utter spendthrift in this regard but tends to build up very considerable temporary reserves during periods of plentiful supply. The point which deserves greatest emphasis is that the period during which an excess can be stored is relatively brief. This is probably in part a reflection of the delicacy of the structure of the thiamin molecule which gives the substance a perishable nature in the body.

Aside from the question of the overall thiamin economy the storage and excretion of thiamin presents many problems which are of potential interest regarding its physiological function. Thus the motive of some of the research in this field has been to find out how thiamin is distributed in the various organs and how rapidly it is depleted in each during a period of deficiency. Hints were sought in this way as to where and how the substance is utilized.

Shortcomings of our methods of assay have operated as serious restraints to progress toward such knowledge and still offer considerable obstacles. These obstacles comprise not only uncertainty as to the specificity of the tests in use but also the laborious and tedious nature of the work which necessarily limits the annual output of results.

Many of the earlier data obtained were unsystematic as to the distribution of thiamin in the body, as the experiments involved

were aimed at determining the potency of foodstuffs from animal sources rather than the fate of the substance in the body. Cooper thus early assayed several of the organs of beeves (1) by feeding the tissues in definite quantities to pigeons which were otherwise maintained on polished rice. He used as a gauge of vitamin content not only the prevention of polyneuritis but also the maintenance of weight. The several organs were rated as follows in descending order of potency: liver, heart, cerebrum, cerebellum, skeletal muscle. Hess and Takahashi followed with evidence of depletion of the substance in the organs of polyneuritic pigeons (2). For this purpose they fed mice and rats on polished rice with addenda of the tissues from normal and from polyneuritic birds, respectively, and found that the former were somewhat more effective in prolonging the lives of the rodents. Abderhalden (3) pursued the subject further by similar methods, using the tissues of pigeons as dietary supplements for pigeons fed on polished rice and also on a basal diet of the more modern type. Again a superiority was observed of normal tissues over polyneuritic ones in respect to prevention of recurrence of polyneuritic symptoms. Among the normal tissues liver was superior, but muscle, kidneys, and brain displayed no significant differences, one from another. The outstanding fact, however, according to Abderhalden's view, was that the organs of polyneuritic birds appeared still capable of affording some protection to the birds which ate them.

Westenbrink has been much impressed by this fact and has repeatedly emphasized the retention of considerable amounts of thiamin in the organs of polyneuritic animals (4). In his study of the matter Westenbrink used a weight-restoration method of assay (Chapter XVII) by means of which relative values were obtained for the several organs in various states of depletion. No attempt was made to convert the values into standard units. Thus were secured, for the first time, some quantitative ideas of thiamin content of organs of rats and of the depletion which they suffer in the course of periods of deficiency of vitamin in the diet. The assays were checked by comparing the result of tissue feeding with those of administering definite doses of pure thiamin.

The animals used in these experiments fall into two groups, namely, the animals which were slaughtered to provide tissues and those which were used for the assay of the tissues. The former consisted of six groups of sixty animals each. Males and females were equally distributed. The first group was fed for five weeks on the following normal diet: ⅓ whole milk powder, ⅔ whole wheat meal with cod liver oil addendum. The remaining five groups were fed on a B_1 deficient diet consisting of approximately 85 per cent washed polished rice, 3 per cent casein,

3 per cent salts, and 10 per cent autoclaved yeast. Cod liver oil was given as an addendum. The animals of one of these five groups were slaughtered after one week on this diet, a second after two weeks, and so on. All animals weighed 150 to 200 grams at the outset of this feeding and underwent no great changes in weight on the diets. Those on the deficient diet for the longer periods were found to be almost depleted of fat. None of the rats developed forthright polyneuritis during the period of feeding. The same thiamin-deficient basal diet was employed for the animals used for assay purposes. After weight loss set in, these rats were given single doses of one of the dried tissues and the rises in weight were observed.

By such means Westenbrink reached the conclusion that the liver loses four fifths of its vitamin during a single week of deficiency and that the loss then progresses more slowly till only about one twentieth of the original content is left at the end of five weeks. The kidney, starting at nearly the same level as the liver, loses at a like rate. The heart, perhaps at the outset a little richer in thiamin than the liver and kidney, loses equally rapidly in the first week, but much less rapidly thereafter.

The brain is of particular interest because of the outstanding character of the nervous symptoms in beriberi. In this organ in the rat the normal thiamin content is lower than in the above-mentioned organs, perhaps half that of the heart. The loss during the first week is scarcely perceptible and does not attain large proportions at any stage. Westenbrink states that at the end of five weeks about one fifth of the original amount is still present, but his curves appear to justify a higher estimate of the percentage retained.

The muscle, lungs, stomach, small intestine, and spleen had lower thiamin contents in normal animals. In animals on deficient diets they exhibited more gradual losses. The relative magnitude of loss during the first week is less marked. Of all the tissues the muscle reaches a nearly complete depletion soonest. The gut in the deficient rat, in view of its depletion of fat, actually appears richer in thiamin than it should. This complication comes into play in equal or greater degree in the case of the pancreas. No thiamin could be detected in the blood by this method. All of the results are graphically summarized in Figure 8, which represents the present authors' interpretation of Westenbrink's original data. For the purpose of converting the data into γ of the thiamin, we resorted to data which have been published in more recent years on the effect of pure thiamin on the weight curves of rats in a similar condition (5). The data should be regarded as only relatively correct, as many interpolations are involved. The general picture, however, correctly reflects Westenbrink:

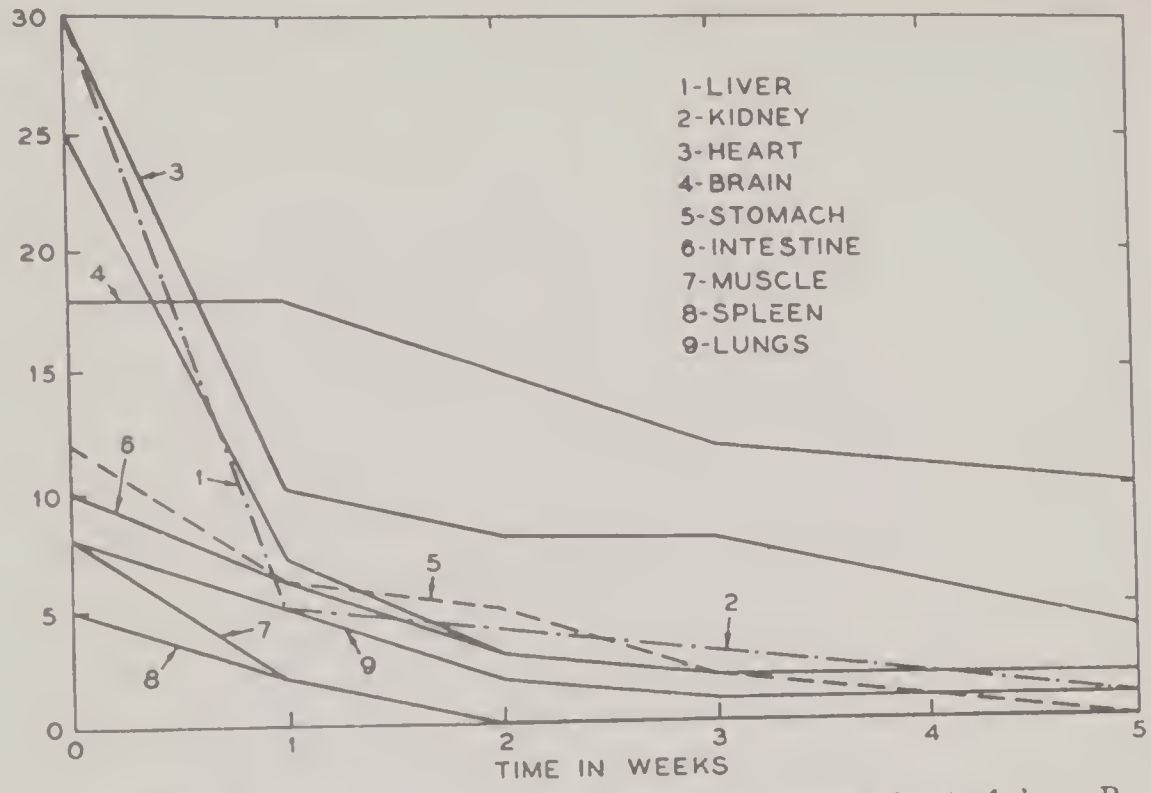


Figure 8. Decline in vitamin B_1 content of organs of rats fed on B_1 deficient diet. The values are expressed in γ of thiamin per gram of dry tissue but are not to be regarded as reliable in an absolute sense.

Westenbrink extended the study to pigeons two years later (6), though with less quantitative detail. The diets and method of assay were the same as were used in the previous study except that autoclaved yeast was omitted from the food of the pigeons used to supply the organs. Table XVII represents the results in a manner convenient for comparison with Figure 8 in order to bring out the resemblances and contrasts between pigeons and rats. The precision of the values is even lower than in the previous figure concerning rats, as fewer assays were made.

TABLE XVII

DECLINE IN THE THIAMIN CONTENT OF TISSUES

Tissue	*Normal*	*13 Days*
Liver	15 γ	5 γ
Heart	15 γ	7 γ
Brain	12 γ	4 γ
Muscle	20 γ	6 γ

Westenbrink summarizes his conclusions somewhat as follows: The muscle in pigeons, in contrast to rats, is higher in thiamin than the liver, kidney, and brain. All pigeon organs lose the greater part of their thiamin within thirteen days on a deficient diet. The initial level in the richer organs of rats is higher than in any of the organs in pigeons but the loss is more rapid at first, so that after two weeks the thiamin contents are similar for the two species. In spite of this fact, pigeons contract polyneuritis much sooner after reaching this stage than rats do. The

more rapid and greater loss in the pigeon brain seems significant. Westenbrink rejects the idea that the occurrence of polyneuritis is conditioned by complete exhaustion of thiamin supply.

A comparison of carbohydrate-rich diet versus high-fat diet as to their effects upon the extent of exhaustion of the thiamin content of tissues of pigeons is also included in Westenbrink's later paper. His conclusion is that the fat content of the diet does not influence the rate of exhaustion of thiamin, a conclusion which the present authors feel is based upon somewhat too slender evidence. (See Chapter XXI, where this subject is discussed in greater detail.) However, it is certainly true that there is little contrast in the effects of the two types of diet.

Kemmerer and Steenbock (7), using essentially the same method as that of Westenbrink, made a special study of the effect of fat in the diet upon the conservation of thiamin in the tissues. The following figures on thiamin content of various tissues are inferred from the tabulations of these authors:

	Tissue	*Diet*	*γ per Gram (Fresh)*
Rat	Muscle	Normal	2
	Muscle	Low B_1	1
	Liver	Normal	12
	Liver	Low B_1	1
Chick	Muscle, dark	Normal	2
	Muscle, dark	Low B_1	1
	Muscle, light	Normal	2
	Muscle, light	Low B_1	1
	Liver	Normal	12
Pork, young	Muscle	Normal	6–8
Pork	Muscle	Normal	12
Beef	Muscle	Normal	1.5

The effect of fat in the diet on the thiamin in the tissues could not be discerned. Liver seems to be prevalently high in thiamin content; muscle to be quite variable from species to species.

Shin (8, 9) has contributed a series of studies of the distribution of thiamin in the tissues of rabbits. He rates the tissues in the following order with respect to thiamin concentration: liver, spleen, kidney, lung, brain, heart muscle, and mammary gland. The aggregate amount in the blood is said to be half that in the liver. Rabbit urine contained a little thiamin, but these authors found none in human urine, suggesting that their method was not very sensitive. In pregnant rabbits the mammary gland is reported as showing higher thiamin content than is the case in non-pregnant animals. The liver and kidney show substantial decreases in pregnancy. The fetus contained one-third as much as the liver; the placenta half as much. The progress toward normal in thiamin distribution after pregnancy is indicated. Puerperal urine is said to contain more thiamin than normal, the increased excretion being thought to be partly, at least, responsible for the frequent occurrence of beriberi during

the puerperium. The amount of the tissues, fluids, etc., necessary to cure polyneuritis in pigeons was used as the criterion of thiamin content.

Griffith (10) depleted rats of thiamin by feeding on diet of casein, sucrose, and salt mixture with addenda of cod liver oil and liver extract. The carcasses of the polyneuritic animals were minced after discarding the paws, stomach, and intestines, and the minced tissue was assayed by the rat growth method in comparison with corresponding tissues of normal rats. Very marked depletion was observed, but the data do not permit a quantitative statement.

All of the foregoing assays which have been cited in this chapter were made under such circumstances that we can scarcely trust them for quantitative purposes except in a relative sense. As we have stated in the previous chapter, weight gain is marked on administration of thiamin to animals which have been kept for some time on a deficient diet. When foods containing thiamin are given to such animals, we believe that other factors besides thiamin content contribute to the weight gains and give falsely high values. The values which we have recorded above should be regarded only as a means of comparing the results of various workers and the relative weight-promoting properties of the tissues in terms of pure thiamin required to produce equal weight responses. The results which follow in this chapter, being obtained by better methods, are quantitatively more trustworthy.

Leong (11) gives us the following tabulation of the distribution of thiamin in various tissues of rats. His method of test was that involving the cure of bradycardia in rats. For rats on the stock diet, which was only moderately rich in thiamin, the results are shown in Table XVIII:

TABLE XVIII

THIAMIN CONTENT OF NORMAL RAT ORGANS

	THIAMIN, γ PER GRAM OF FRESH TISSUE			AV.	THIAMIN, γ IN ENTIRE ORGAN		
	A ♂	B ♀	C ♂		A ♂	B ♀	C ♂
Body weight	152	160	240	—	152	160	240
Liver	4.5	5.1	4.2	4.5	7.5	8.2	11.9
Heart	3.9	3.9	4.8	4.2	2.7	2.7	3.0
Kidney	1.2	0.6	1.8	1.2	1.8	0.9	2.4
Brain	3.6	2.7	6.0	4.2	4.8	4.2	7.2
Spleen	3.6	3.0	5.4	3.9	5.4	3.6	5.4
Lung	1.5	1.8	3.6	2.4	1.2	1.8	3.6
Muscle	1.2	1.2	1.5	1.2	57.6	61.2	72.0
Body fat	1.5	0.6	—	1.2	7.5	3.6	—
Blood	—	—	0.9	—	—	—	2.7
				Total	103.5 γ	102.3 γ	137.4 γ

For rats on diets rich in thiamin the liver and heart reached levels of about 7.8 γ and 8.1 γ per gram, respectively, *i.e.*, scarcely twice as high as in the normals. The kidney was nearly three times as high, but other organs showed rather lesser increases. Rats kept for a month on a thiamin-free diet showed 1.8 γ and 0.3 γ per gram in the liver and muscle, respectively; those on average diet 5.1 γ and 1.2 γ, respectively; those on high thiamin diets 7.8 γ and 1.8 γ respectively. The liver and muscle contain 80 to 90 per cent of the whole body store; the liver about 30 per cent; the muscle about 50 per cent.

These results are in rather marked contrast in some respects to those of Brodie and MacLeod (12). These authors concluded on the basis of assays by the rat growth method that there is ten times as much thiamin per gram in the rat liver as in the muscle. Kidney was about half and brain one-third as rich per gram as liver. Heart was almost as rich as liver, while blood, spleen, and lungs contained only traces. This represents the case for animals on a normal diet. On a B_1 deficient diet the tissues, except the brain, became so depleted that thiamin could not be detected by the method used. The tissues of animals on a thiamin-rich diet containing whole yeast showed somewhat higher than normal thiamin contents.

Jarussowa (13) devoted particular attention to the question of temporary storage in pigeons. Various products rich in thiamin were fed in liberal quantities to the birds. Following this, polyneuritis was developed in them by feeding them exclusively on autoclaved buckwheat. The time of onset of polyneuritis and the duration of life were roughly proportional to the quantities of thiamin-rich food previously ingested, indicating a significant capacity of the animals to store thiamin for a few weeks. The present authors can fully confirm this finding from their own experience. Indeed the whole experience of many workers on thiamin assaying both with birds and mammals shows the necessity of a depletion period before thiamin deficiency becomes apparent. This is a sufficient evidence in itself of the capacity for temporary storage.

A very impressive contribution on the thiamin content of blood is due to Meiklejohn (14). Using the weight of mycelium of the fungus *Phycomyces Blakesleeanus*, produced under standard conditions, as the measure of thiamin present, he has adapted the method for use with a few cubic centimeters of blood. The blood of normal pigeons by this method contained about 0.30 γ per cubic centimeter; that of B_1 deficient birds about 0.055 γ. Three specimens of sheep's blood ranged from 0.130 γ to 0.187 γ, while six human cases ranged from 0.7 γ to 0.88 γ per cubic centimeter. Some of the human cases were evidently in pathological states, but no description is given, as the paper deals only with method as primary subject matter.

Cancer tissue is said to be subnormal in thiamin content (15).

Excretion of Thiamin

Excretion in the feces has not generally been regarded as very significant because feces may include unabsorbed residues of thiamin originating in the food and because synthesis of thiamin may occur in the intestinal tract. (See Chapters XVII and XXI.) We will touch upon this phase of excretion only incidentally in the course of discussing the excretion in the urine, a feature which is assuming increasing importance as a diagnostic test of thiamin deficiency. The advent of more adequate and convenient methods of assay is to a large extent responsible for the rising interest in this field.

Early reference was made to the subject by Cooper in 1914. O. M. Helmer made one of the first serious attempts to put the matter on a quantitative basis (16). He evaporated human urine *in vacuo* and fed the residue in admixture with hydrogenated cottonseed oil to rats which had been kept on a thiamin-deficient diet until the weight began to decline. He was able to show a difference in vitamin content of the urine between normally fed individuals on the one hand and, on the other, one patient exhibiting evidence of a deficiency state. Roscoe (17) followed an essentially similar technique with similar results. Thiamin was not found in urine of subjects on the hospital diet but was encountered when excess vitamin was fed. When about 2.5 mg. of thiamin was administered as a yeast concentrate, one fifth to two fifths of that amount appeared in the urine. A patient with alcoholic neuritis seemed to excrete a slightly larger proportion than did the normals.

The development of the bradycardia method of testing seems to have contributed very markedly to the progress of our knowledge on this subject. This method requires some further critical study before its results for certain types of assays can be accepted with finality. (See Chapter XVII.) However, it seems peculiarly well adapted to study of urinary output, and the results seem remarkedly consistent in this case. In this instance relative values are more important than absolute values, as under ordinary circumstances it appears that only a small and somewhat variable percentage of the total intake is excreted in the urine.

The first results on human urine obtained with this method are reported by Harris and Leong (18). Nine healthy adults of 17 to 37 years of age on "normal" diets excreted 30 to 90 γ of thiamin in the urine daily. The average was 60 γ. This was estimated to represent about 5 to 8 per cent of the thiamin in-

gested. As a tentative standard it was proposed that less than 36 γ per day excreted should be regarded as an indication of a B_1 deficiency. Preliminary tests indicated that in beriberi the excretion may fall to 7 to 8 γ daily. (See p. 102.)

One of these authors has contributed much more voluminous data on excretion by rats in a later paper (19). This study includes the matter of fecal excretion and bacterial synthesis. The latter is regarded as small enough as to cause no serious difficulty in interpretation of results. Normal rats excreted 1.5 to 4.2 γ per day in the feces. When given 10 to 20 International Units of standard adsorbate they excreted 2.7 to 5.4 γ the following day in the feces. Thiamin-deficient rats excreted 1.5 to 4.2 γ in the feces; when given 14 to 200 mg. of standard clay, their fecal excretion for the following day covered the same range, but surprisingly averaged substantially lower, viz., 2.4 γ versus 1.2 γ. The reduced excretions were from those animals which received the larger amounts of adsorbate, viz., 200 mg. It is concluded that very little thiamin escapes absorption in the gut. When much larger amounts of clay were administered, an excess was found in the feces. In general, fecal excretion is regarded as small and substantially constant over the usual range of intakes.

Rats on ten different diets ranging in thiamin content from 0 to 112.5 γ per gram were used for the study of urinary and fecal output of thiamin. Thiamin was given in the form of the International Standard Clay. The adsorptive properties of the clay may have influenced excretion especially at high levels of intake. The results, shown in Figures 9 and 10, are quite striking. With low intakes the urinary output was low; at high intakes it remained constant at about 270 γ per day. This is, of course, a very large amount in view of the fact that the normal daily intake of the rat probably lies in the range of 15 to 30 γ. The fecal output was low till the intake rose above 300 γ daily.

Injection of 750 γ of thiamin in solution led to excretion of from 390 to 660 γ in the urine of two normal rats, but no more than 6 γ in the feces.

At the end of the experiment on the above series of ten diets the body stores of thiamin were determined by the same method. These are set forth in Table XIX.

From this tabulation it is seen that at levels of from 10 to about 40 γ no increase in stores took place, and substantial amounts of thiamin up to about 2.5 mg. must have been destroyed in the body of each rat during the course of the experiment. Better growth was obtained on still higher levels of thiamin up to about 100 γ per day, and some accumulation of stores began to appear in that range. Considerable interest attaches to these observations as confirmatory of the viewpoint

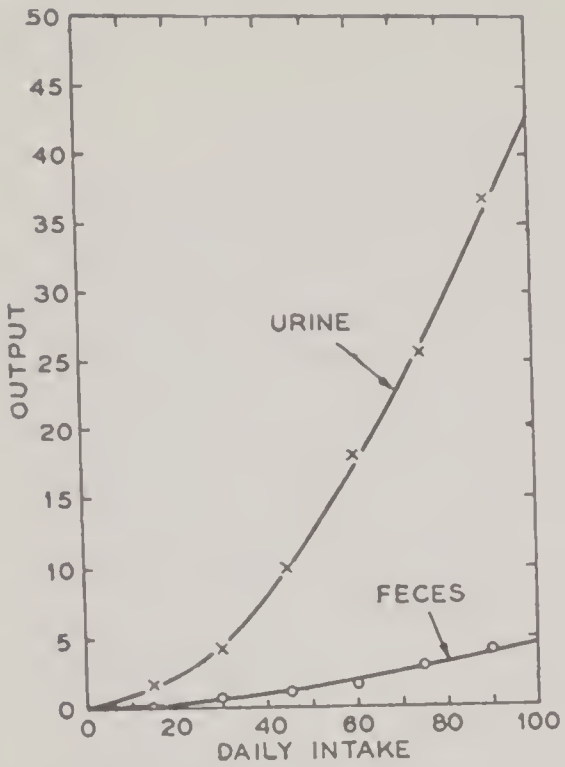


Figure 9. Relation of thiamin intake to excretion at low levels of intake. The values are expressed as γ of thiamin.

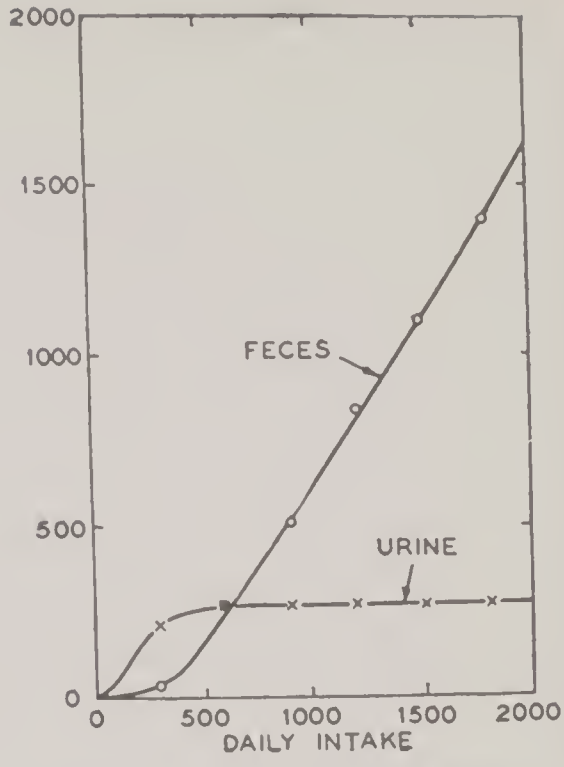


Figure 10. Relation of thiamin intake to excretion at high levels of intake. The values are expressed as γ of thiamin.

TABLE XIX

Thiamin Balance Sheet

Group	Rat	Intake, γ per Day	Intake Minus Output, γ	Days on Experimental Diet	Weight Change	Stores in Body, γ	Loss or Gain in Stores during Experiment, γ
I	1	0	− 0.3	20	− 52	3.9	− 101
	2	0	− 0.6	20	− 37	3.9	− 101
II	3	2.7	+ 2.4	57	− 31	13.5	− 93
	4	2.7	+ 2.4	57	− 38	7.5	− 99
III	5	8.7	+ 7.1	80	+ 28	72	− 33
	6	9.3	+ 8.1	58	+ 23	72	− 33
IV	7	20.1	+ 18.0	66	+ 55	90	− 15
	8	23.4	+ 22.2	80	+ 117	126	+ 21
V	9	39.3	+ 28.8	68	+ 66	123	+ 18
	10	41.4	+ 30.9	63	+ 40	114	+ 9
VI	11	82.5	+ 38.4	63	+ 55	177	+ 72
	12	103.5	+ 61.5	67	+ 121	267	+ 162
VII	13	261	+ 93	63	+ 125	354	+ 249
	14	333	+ 63	76	+ 139	231	+ 126
VIII	15	543	+ 102	44	+ 107	258	+ 162
	16	591	+ 69	50	+ 99	252	+ 147
IX	17	891	+ 153	46	+ 57	243	+ 138
	18	1464	+ 66	40	+ 78	258	+ 153
X	19	1464	+ 42	32	+ 66	240	+ 135
	20	2112	+ 153	32	+ 33	231	+ 126

of Ammerman and Waterman that animals are able to profit to a considerable degree by more than usually generous supplies of thiamin (see Chapter XXII). At the still higher levels there is not only considerable wastage in the urine but also some 90 γ per day are destroyed per rat.

Knott (20) attempted to ascertain the thiamin requirement of young children by determining the proportion excreted in urine and feces at several different levels of thiamin intake. Increasingly high retentions were observed with higher levels of ingestion, and the optimum utilization corresponded to a level of about 100 γ of thiamin per day per kilo of body weight. This study assumes that the thiamin not excreted is utilized or stored and does not take into account destruction in the body. This error is important from a theoretical standpoint but probably not from a practical one as far as human nutrition is concerned, since economy of children is rated as more vital than economy of thiamin.

A suggestive observation by Bernstein (21) on the excretion of vitamin C in sweat raises the question as to whether this form of excretion may not be an important source of loss of thiamin also. This is true especially because hard labor has often been regarded as conducive to beriberi. Bernstein found that mine laborers lost as much as 2 mg. per hour of vitamin C in sweat and displayed frequent evidence of scurvy even when the diet was quite adequate in this regard according to ordinary standards.

Summary

Storage of thiamin is widespread in the body, the highest concentrations being in liver, kidney, heart, and brain. The concentration in the muscles is low in the rat, the young chick, and the ox, but is substantially higher in the adult hog and somewhat so in the adult pigeon. The evidence suggests that it is usually higher in the musculature of the adult than of the young. The aggregate of muscle and liver thiamin constitutes the larger part of the body store.

On deficient diets the amounts stored decline rapidly at first, then more slowly, the last minimal amounts being retained quite persistently. Depletion appears to proceed most rapidly to low levels in the musculature; the brain appears to retain its thiamin to a great extent even at the expense of other tissues.

On thiamin-rich diets the total storage rises appreciably so that the effects of high intakes during a short period may persist for a few weeks but not for long periods. Excess vitamin is largely wasted in excretion or by destruction in the body.

The fecal excretion is fairly constant and relatively small in rats. The urinary excretion in both rats and humans is quite sensitive to the intake in the food. Although only a small percentage of the intake is excreted in the urine, change of intake is promptly and markedly reflected by the urinary output. A large amount of work is now in progress, showing that urinary output of thiamin is a useful and reliable index of deficiency states. Much of it is not yet ready for publication but doubtless will appear shortly. There is a great need for a study of thiamin distribution in human tissues. Only a few scattered data have appeared.

BIBLIOGRAPHY

1. Cooper, A. E.: On the Protective and Curative Properties of Certain Foodstuffs against Polyneuritis Induced in Birds by a Diet of Polished Rice. Jour. Hyg. *14*, 12, 1914.
2. Hess, W. R., and Takahashi, N.: Nachweis eines stofflichen Defizits in Gewebe an Avitaminose erkrankter Tiere. Biochem. Zeit. *122*, 193, 1921.
3. Abderhalden, E.: Studien über das Wesen der Wirkung des Vitamin B Komplexes. I. Mitt. Pflüger's Arch. *226*, 723, 1931.
4. Westenbrink, H. G. K.: Über den Gehalt an Vitamin-B_1, der Organe von weissen Ratten bei normaler und Vitamin-B_1-freier Ernährung. Arch. Neerl. *17*, 560, 1932.
5. Jansen, B. C. P.: Determination of the Value of the International Standard. Zeit. f. Vitaminforsch. *5*, 254, 1936.
6. Westenbrink, H. G. K.: Über das Verschwinden des Vitamins-B_1 aus einigen Organen von Tauben bei Vitamin-B_1-freier, Kohlehydratreicher, Fettarmer Nahrung, Bezw. bei Vitamin-B_1-freier, Kohlehydratfreier, Fettreicher Nahrung, nebst einer Vergleichung des Vitamin-B_1-Gehaltes von Organen von Ratten und Tauben. Arch. Neerl. *19*, 116, 1934.
7. Kemmerer, A. R., and Steenbock, H.: Study of the Sparing Action of Fats on the Vitamin B Content of Animal Tissues. Jour. Biol. Chem. *103*, 353, 1933.
8. Shin, H.: Studies on Pregnant, Puerperal and Infant Beriberi. Jour. Chosen Med. Assn. *22*, 1011, 1932.
9. Shin, H.: Studies on Pregnant, Puerperal and Infant Beriberi. IV. Vitamin B Content of Various Organs and Secretions Including Blood, Urine and Embryo from Pregnant or Unmated Rabbits Fed on Vitamin B Deficient Diets. Jour. Chosen Med. Assn. *23*, 242, 1933.
10. Griffith, W. H.: Studies of Growth. B and G Content of Body

Tissues of Normal and Experimental Rats. Jour. Nutrition, *10,* 675, 1935.

11. Leong, P. C.: Vitamin B_1 in the Animal Organism. I. The Maximum Storage of B_1 in the Tissues of the Rat. Biochem. Jour. *31,* 367, 1937.
12. Brodie, J. B., and MacLeod, F. L.: Quantitative Experiments on the Occurrence of Vitamin B in Organs. Jour. Nutrition *10,* 179, 1935.
13. Jarussowa, N. S.: Zur Frage über die Anhaufung des antineuritischen Vitamins (B) im Organismus von Tieren. Zeit. f. Vitaminforsch. *6,* 98, 1937.
14. Meiklejohn, A. P.: Estimation of Vitamin B_1 in Blood by a Modification of Schopfer's Test. Biochem. J. *31,* 1441, 1937.
15. Nakahara, W., and Somekawa, E.: Vitamins and Tumour Growth. Vitamin B Content of Rat Tumours. Proc. Imp. Acad. (Japan) *6,* 345, 1930.
16. Helmer, O. M.: Vitamin B_1 and B_2 Content of Human Urine. Proc. Soc. Exp. Biol. Med. *32,* 1187, 1935.
17. Roscoe, M. H.: B Vitamins in Human Urine. Biochem. J. *30,* 1053, 1936.
18. Harris, L. J., and Leong, P. C.: Excretion of Vitamin B_1 in Human Urine and Its Dependence on Dietary Intake. Lancet *1,* 886, 1936.
19. Leong, P. C.: Vitamin B_1 in the Animal Organism. II. A Quantitative Study of the Metabolism of Vitamin B_1 in Rats. Biochem. J. *31,* 373, 1937.
20. Knott, E. M.: A Quantitative Study of the Utilization and Retention of Vitamin B by Young Children. Jour. Nutrition *12,* 597, 1936.
21. Bernstein, R. E.: Excretion of Vitamin C in Sweat. Nature *140,* 684, 1937.

CHAPTER XX

THIAMIN REQUIREMENT IN RELATION TO QUANTITY OF FOOD

Westenbrink has given us an excellent review of a large part of the existing literature upon this subject. This review has been drawn upon freely in the discussion which follows. While there is some conflict in the evidence, on the whole it provides a definite support for the thesis that the B_1 requirement rises with the food consumption.

The experiments of Cooper (2) involved forced feeding of pigeons upon various quantities of polished rice ranging from 1/11 to 1/30 of the body weight daily. The number of birds at each feeding level was small, so that the comparative results on the forcibly fed birds cannot be considered statistically. On their face the figures do not appear to show any relationship within the range of experiment between the amount of food which was administered and the rapidity of development of polyneuritis. The group of forcibly fed birds as a whole, however, developed polyneuritis in a substantially shorter time than was the case with the parallel series of birds which were allowed to limit their food intake according to their appetites. Thus the time elapsing before polyneuritis in the case of the forcibly fed birds ranged from 9 to 26 days; the majority succumbed at 13 to 16 days. By contrast the birds fed *ad libitum* withstood polyneuritis from 21 to 33 days, the mean being about 26 days. Under the latter circumstances, as all experimenters are well aware, the food consumption of pigeons which may amount at first to 1/20 of the body weight rapidly falls as the animals are depleted of vitamin until, during the last several days of life, food consumption may drop to 1/200 of the body weight or less. It is of great interest to note that all animals thus protect themselves from polyneuritis in some degree by self-starvation, the appetite declining as depletion advances. It has often been remarked that birds in this condition eagerly seek out any discolored grains, perhaps in search of those which retain a certain amount of bran.

In the foregoing experiments Westenbrink notes that the rice was presumably not entirely free from thiamin so that the

forcibly fed birds automatically received more thiamin as they received more food; conversely the free-eating birds automatically lessened their thiamin intake as the experiment progressed and their food consumption fell. The disparity of thiamin intake of the two groups was thus enhanced and the true increase of the thiamin requirement with increased food intake is somewhat greater than that indicated by the comparative times of onset of symptoms.

Braddon and Cooper (3) fed six chickens on 30 grams (1/20 of the body weight) daily of paddy (whole rice retaining the hulls as well as bran). These remained healthy for 45 days. Six chickens fed an equal amount of paddy, plus 90 grams of well-washed polished rice daily, exhibited polyneuritis in 10 to 16 days. In another similar experiment it was shown that 22½ grams of paddy alone kept chickens healthy for 30 days, but that the forced feeding in addition of 54 grams of washed polished rice produced polyneuritis in 20 to 30 days. Also, five chickens were fed polished rice to the extent of 1/5 of the body weight daily for 5 days and thereafter 1/20 of the body weight. They developed polyneuritis in 15 to 20 days. When the feeding was continued at the 1/5 level in another group, polyneuritis occurred in from 11 to 15 days.

These experiments, particularly the latter ones, have been questioned by Vedder (4) on the ground that the amount of food fed was grossly excessive. Vedder found it possible to kill fowls by feeding them with such quantities of brown rice where no question of vitamin deficiency could be involved. Vedder, feeding other fowls *ad libitum* on polished rice, was able to note no correlation between the rapidity of onset of polyneuritis and the amount of food consumed. The present authors have also confirmed by experiment the physical impossibility of feeding pigeons continuously with as much as 20 grams of polished rice daily except when ample vitamin is supplied. The crops soon become gorged with undigested food which ferments and is often regurgitated. Several instances of burst crops were encountered. It seems incredible that the amounts of food which have been reported as having been given to birds in many experiments in the literature can actually have been given and retained. Some concession must have been made to the overstuffed animals after the lapse of a few days. Unfortunately animal feeding is often entrusted to ill-trained and not overly conscientious attendants.

Braddon and Cooper also experimented with pigeons which were reported to have been fed daily from 1/10 to 1/25 of their body weights of washed polished rice, supplemented by quantities of yeast, ranging from 1/3500 to 1/2500 of the body weight. With the larger amounts of food polyneuritis ensued more

quickly in spite of B_1 supplied by the yeast. A few similar experiments with chickens gave similar results.

Funk (5) forcibly fed pigeons with polished rice in amounts of 5, 10, and 20 grams daily, polyneuritis ensuing on the average in 39, 36, and 22 days, respectively. Evidently there is a significant difference between the last two mentioned groups, but only a very doubtful one between the first two groups. Here again Vedder's criticism seems to have validity, since 20 grams of food is more than pigeons of ordinary size can safely consume over a long period of time, especially when partially avitaminotic. In Funk's experiments one must also presume that some thiamin was present in the rice and that the vitamin intake rose in proportion to the food.

A modified experiment was performed by Jansen (6). Pigeons were fed upon a diet consisting of 90 parts of polished rice and 10 parts of mungo beans (*Phaseolus mungo*), receiving 12 grams daily of this mixture. They survived for variable periods ranging from 3 to 18 weeks, the average survival being approximately 9 weeks. Another group of pigeons receiving 24 grams daily of the mixture survived from 2½ to 6 weeks; average 4 weeks.

All the foregoing experiments involve overfeeding in greater or less degree. Pilcher and Sollman (7) found that even with very large daily supplies of B_1 (960 milligrams of Harris yeast vitamin powder) pigeons of 260–460 grams' weight did not voluntarily consume more than 15 to 17 grams of polished rice daily.

Plimmer, Rosedale, and Raymond (8) reached the conclusion that

$$\frac{B_1 \text{ Requirement}}{\text{Calorie Intake}} = \text{Constant}$$

as a result of experiments performed by them. Without reviewing these experiments, which are not very convincing in character, we can agree with the conclusion of Westenbrink that this relationship requires modification if we accept results such as those of Jansen and Funk which are cited above. Otherwise it would not be possible to hasten the onset of polyneuritis and death by increasing the intake of a vitamin-containing food, since in such experiments the vitamin intake rises exactly in proportion to the food intake. Westenbrink suggests a modified expression of the relationship as follows:

$$\frac{B_1 \text{ Requirement}}{(\text{Calorie Intake})^x} = \text{Constant}$$

in which x has a value greater than 1. No attempt is made to assign a precise value to x. As this formula applies only to an

individual animal at different levels of feeding under highly artificial conditions ranging from semi-starvation to gross overfeeding, it must not be confused with the natural variation of food intake and of B_1 requirement which results from variation in size of individuals, the degree of their muscular activity, species differences, etc. These are treated in later chapters.

J. C. Keresztesy, in the authors' laboratory (unpublished results), performed some rather striking experiments which appear to eliminate any question of mechanical injury by forced feeding. They also have the advantage of keeping the food intake well within the range of normal voluntary consumption by healthy animals. He fed twelve pigeons on polished rice and oyster shells *ad libitum,* together with 1 gram daily of meat extract and salt mixture. After 21 days' depletion on this diet they were given in addition a dose on alternate days of 8 milligrams of an activated clay similar to International Standard Clay though about twice as potent. With this amount of clay the animals reached a constant weight level at about 25 per cent below normal. As determined by supplementary experiments, such birds are precisely in balance, that is, they promptly decline in weight and incur polyneuritis if the dosage is lowered. It was now shown that these birds likewise promptly succumbed to polyneuritis when the food intake was increased by forced feeding of 12 grams of rice daily, although the vitamin addendum remained unchanged. They recovered again in those instances in which the clay intake was increased to 20 milligrams on alternate days. At this level of vitamin intake several of them were able to tolerate 12 grams of polished rice very well for weeks. Details of these experiments are given in Figure 11.

While these results provide convincing evidence that the B_1 requirement is enhanced by increased food intake, one cannot safely estimate the value of x in Westenbrink's formula indicated above. Indeed there appears to be no sufficient reason for believing that x is a precise constant, even through the relatively narrow range of normal feeding, much less through the wide extremes from semi-starvation on the one hand to overfeeding on the other. The precise value of x has no great practical importance as the range of undersupply or oversupply of food which an animal can long tolerate is relatively narrow. The theoretical importance of the fact that $x > 1$ is considerable, as it indicates that B_1 has to do with the metabolism of food, since the effects of a B_1 deficiency are aggravated by overloading the metabolic machinery.

It should be noted that all of the above animal experiments involve birds on highly starchy diets. No similar experiments on mammals have been encountered nor have similar bird ex-

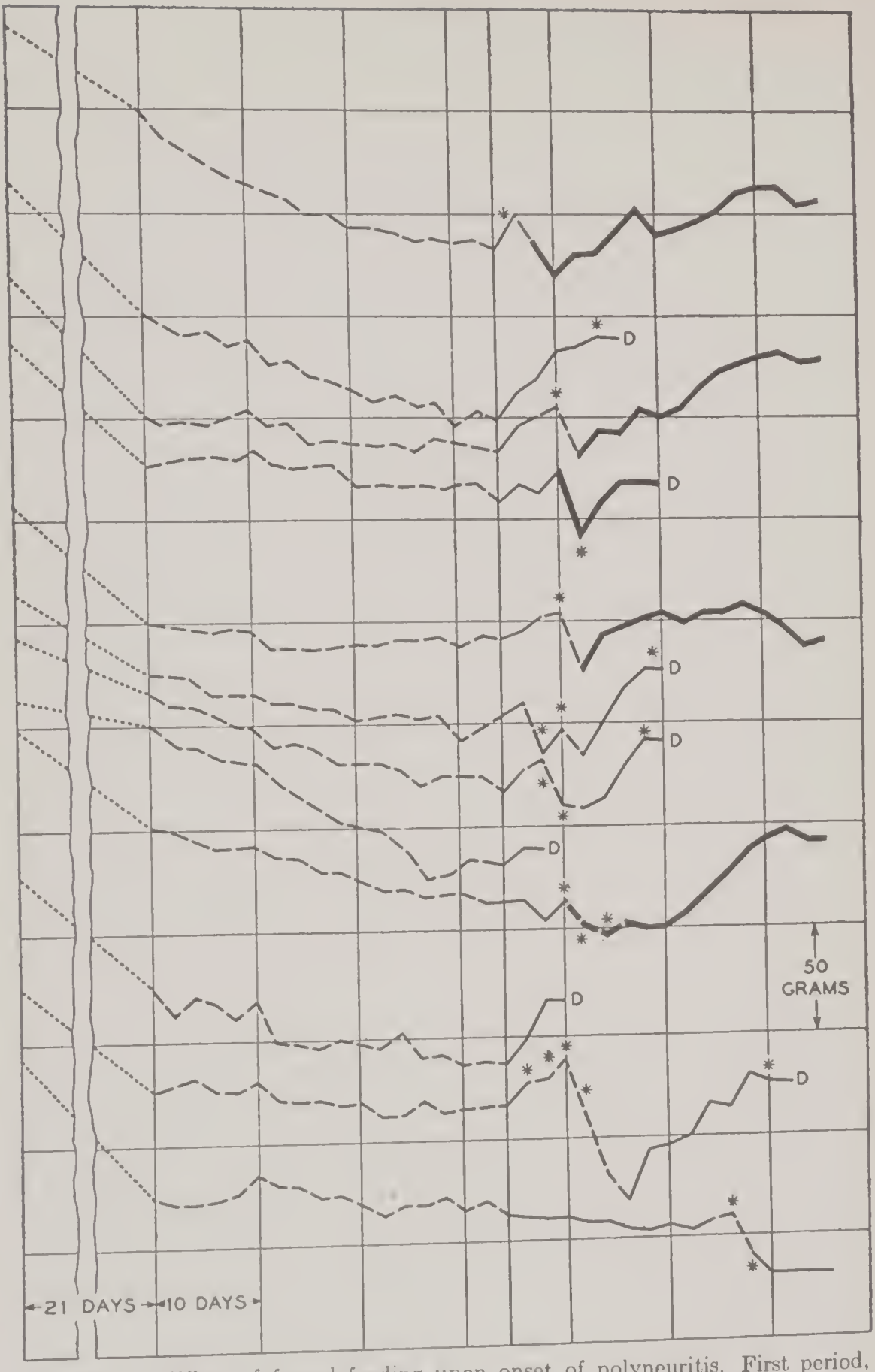


Figure 11. Effect of forced feeding upon onset of polyneuritis. First period, 21 days polished rice only *ad libitum*. Second period, 34 days polished rice *ad lib.* plus 4 mgs. adsorbate. Third period, solid lines indicate forced feeding of 12 gms. of rice daily supplemented by 4 mgs. adsorbate (light lines) or 10 mgs. adsorbate (heavy lines). Forced feeding discontinued during periods indicated by broken lines. * indicates polyneuritic symptoms; D—death.

periments extended to high fat or high protein diets so far as the authors are aware.

Westenbrink (9) reported development of polyneuritis in pigeons in substantially the same length of time whether each received 3 cc. daily or 9 cc. daily of peanut oil in addition to 3 grams of a mixture of casein, salts, and cod liver oil. Although this experiment is not very convincing because only two levels of feeding were involved, it indicates that increasing caloric intake by addition of fat does not increase the thiamin requirement. Other phases of the effect of fatty diets are discussed in the next chapter.

Metabolic Rate

In view of what has been said of the dependence of the thiamin requirement upon the food intake, it is not surprising that the thiamin requirement also appears to be related to the metabolic rate. Cowgill (10), and Cowgill, Rosenberg, and Rogoff (11) studied the effect of exercise on the requirement. Dogs were maintained on a vitamin B deficient diet consisting of 41.2 per cent casein, 29.4 per cent sucrose, 18.3 per cent lard, 7.2 per cent butter, 2.6 per cent bone ash, and 1.3 per cent salt mixture. The weight of each animal, its average food consumption, and the time required to develop anorexia were noted (Table XX). The dogs were then restored to normal by administration of a "vitamin B" concentrate (Harris yeast powder) for some time, and the experiment was repeated with the modification that they were given 45 to 90 minutes exercise in a treadmill daily. As seen in the table, the time required to develop anorexia was reduced by one third to one half, evidently as a result of the exercise.

TABLE XX

The Effect of Exercise on the Time Required for the Development of Anorexia in Dogs

Dog	Body Weight When		Food Intake Daily When		Duration of Daily Exercise	Time on Deficient Diet Required for Development of Anorexia When		Ratio $\frac{y}{x}$
	Not Exercised	Exercised	Not Exercised	Exercised		Not Exercised x	Exercised y	
	kg.	*kg.*	*grams*	*grams*	*minutes*	*days*	*days*	
C	7.8	7.8	118	128	45	21	13	0.61
I	11.4	10.5	135	200	90	21	11	0.52
F	12.0	12.0	183	193	45	18	10	0.55
G	12.0	12.3	180	250	90	18	8	0.44
D	13.7	13.8	150	200	90	24	14	0.58

During a subsequent period it was demonstrated that an adequate supply of "vitamin B" permitted the dogs to consume more food without ill effects and to undergo the exercise without loss of weight.

In other experiments hyperthyroidism was induced in four dogs by the administration to each of 5 grams of desiccated thyroid daily while they were fed on the above-described basal diet with a liberal "vitamin B" addendum. The food consumption rose to about twice that of normal dogs of similar size on the same diet. The addendum was discontinued and typical anorexia developed in an average of seventeen days, accompanied by an appreciable weight loss. In the absence of thyroid feeding the average time to develop anorexia in the same dogs was thirty-two days.

In experiments with pigeons it was found that increased amounts of "vitamin B" were required to maintain appetite and body weight when thyroid was administered.

Sure and Smith (12) find that rats are protected to a considerable extent from the toxic effects of 0.03 to 0.06 mg. of thyroxine per rat per day by ample supplies of vitamin B complex. Larger amounts of thyroxine up to 0.5 mg. per day, however, kill the animal in spite of liberal supplies of B. In 1937 Sure and Buchanan (13) extended the study to pure thiamin. Sixteen groups of four rats each, litter mates of the same sex, received graduated daily doses of 0.05 to 0.2 mg. of thyroxine with daily doses of thiamin ranging from 1 to 160 γ while being maintained on an otherwise thiamin-deficient diet. Judging from the comparative gains and losses in weight of the individuals given (*a*) no addendum, (*b*) thyroxin, (*c*) thyroxin and thiamin, (*d*) thiamin, the authors calculated the per cent protection against thyroxin afforded by various doses of thiamin. Some representative figures are:

Thyroxin *mg.*	*Thiamin* γ	*Per Cent Protection*
0.05	3–5	40–48
0.10	20	42
0.20	80	0
0.20	160	32

Using another ration in which yeast was incorporated, the protection against thyroxin was much better. Supplementary doses of 30 to 100 γ of thiamin afforded 64 to 94 per cent protection against daily doses of 0.2 mg. thyroxin.

From these studies we are justified in accepting the conclusion that an increased metabolic rate increases the thiamin requirement. Some fragmentary evidence from the literature of beriberi seems to support such a view. Thus Egan (14) points out that in an epidemic of beriberi on shipboard the stokers were

affected more seriously than the sailors in the ratio of 34 to 3. This may well have been due directly to the greater physical exertion of the former resulting in a higher food consumption. Whether or not such higher food consumption in such a case would result in a proportionately higher vitamin intake would depend on whether or not their physical exertion led them to select their food differently. As the principal high caloric element of the ration was rice, the hard-working men might well be led to eat relatively more of this more deficient component of the diet.

Clearly, if there is a relationship between food consumption and thiamin requirement, it should be reflected in the history of beriberi and in the character of the food intake associated with outbreaks of the disease. These and related matters are discussed at some length in Chapter VII.

In the literature of beriberi epidemics in Japan, Burma, Siam, and elsewhere a great preponderance of beriberi in males is noted. This may be due to a considerable degree to the higher metabolic rate of males and especially to the harder physical work to which men are customarily subjected. It is doubtless partly due to the fact that many of these epidemics have been associated with labor camps, military organizations, jails, etc., where men predominate and where restriction to a systematic ration occurs to an extent that is not the case in individually managed homes. However, the predominance of males among beriberics is distinctly contrary to the author's own experience in Manila. There, over a period of several years of experimentation with human beriberi (15), by far the most abundant cases were those of mothers and nursing infants. Possibly this is due to relatively less arduous physical exertion of males among urban Malays in the tropics than is the case with other Oriental peoples in general; possibly a lesser proportion of the milder forms of beriberi have been included in reports of epidemics from other Oriental lands, and if attention were confined to severe beriberi, a different distribution between the sexes in Manila would have been observed. The high incidence of mild beriberi among women of childbearing age in Manila was very striking. It furnishes further evidence that the metabolic rate is of importance in determining the B_1 requirement, the rate being substantially heightened during the later months of gestation (16) and during lactation (see Chapter XXII).

At this point it may be appropriate to comment on the relatively low incidence of beriberi among children after the weaning age. This may be ascribed to the fact that children have additional degrees of freedom of accommodation to a deficient diet. In the presence of a deficiency of thiamin, food consumption is lessened. This reflects itself in the child either in lowered

growth or decreased playful activity and only ultimately in loss of flesh. The adult has no growth impulse to be sacrificed, continued activity is forced by economic necessity, and as loss of flesh necessarily follows lowered food intake, the urge to eat drives the victim on toward beriberi. In the child the deficiency presumably first stunts the growth as it does in young rats, the the beriberic condition developing much later.

In fevers the metabolism rises (17), and it seems not unlikely that the extensive association of beriberi with malaria and tropical fevers (18) is due to this cause. Particularly in Brazil there has been a marked reluctance to accept the vitamin hypothesis of the etiology of beriberi and to prefer to ascribe to it an infectious cause because of the association of the disease with fevers (see Chapter II).

Summary

The absolute amount of thiamin required by an individual under ordinary circumstances rises in proportion to the food (or carbohydrate) metabolized.

When, however, the metabolic machinery is strained, as in the case of forcible feeding, above the demands of the appetite, the quantity of vitamin required per calorie of food shows a distinct tendency to rise.

A similar rise in quantity of vitamin required per calorie of food is induced by exercise, by hyperthyroidism, or by the physiological strain of gestation and lactation.

A metabolic function of the vitamin is clearly indicated.

BIBLIOGRAPHY

1. Westenbrink, H. G. K.: Über den Einfluss der Menge und der Zusammenstellung der Nahrung auf die Folgen von Vitamin-B_1 Mangel. Arch. Neerl. de Physiol. *19*, 94, 1934.
2. Cooper, A. E.: On the Protective and Curative Properties of Certain Foodstuffs against Polyneuritis Induced in Birds by a Diet of Polished Rice. Jour. Hyg. *12*, 436, 1912.
3. Braddon, W. L., and Cooper, E. A.: The Influence of Metabolic Factors in Beriberi. Jour. Hyg. *14*, 331, 1914.
4. Vedder, E. B.: Is the Neuritis-preventing Vitamine Concerned in Carbohydrate Metabolism? Jour. Hyg. *17*, 1, 1918.
5. Funk, C.: Studien über Beriberi. Ztschr. f. physiol. Chem. *89*, 378, 1914.
6. Jansen, B. C. P.: On the Need of the Animal Organism of Anti-beriberi Vitamin and on the Amount of This Vitamin in Various

Foodstuffs. Mededeel. Burgerl. Geneesk. Dienst. Ned. Indie, p. 1, 1923.

7. Pilcher, J. D., and Sollman, T.: Deficient and Surplus Consumption of Vitamin B. Jour. of Pharm, *26*, 203, 1925.
8. Plimmer, R. H. A., Rosedale, J. L. and Raymond, W. H.: Balance of Food by Vitamin B. Biochem. J. *21*, 913, 1927.
9. Westenbrink, H. G. K.: Über die Physiologische Wirkung des Vitamins B_1. Arch. Neerl. Physiol. *20*, 481, 1935.
10. Cowgill, G. R.: Vitamin B Requirement of Man. Yale University Press, New Haven, 1934. Chap. VIII.
11. Cowgill, G. R., Rosenberg, H. A., and Rogoff, J.: Studies in Physiology of Vitamins; Effect of Exercise on Time Required for Development of Anorexia Characteristic of Lack of Undifferentiated Vitamin B. Amer. Jour. Physiol. *98*, 589, 1931.
12. Sure, B., and Smith, M. E.: Hyperthyroidism and Nutrition. I. Vitamin B and Thyroxine. Jour. Nutrition *7*, 547, 1934.
13. Sure, B., and Buchanan, K. S.: Antithyrogenic Action of Crystalline Vitamin B. Jour. Nutrition *13*, 513, 1937.
14. Egan, P. B.: An Outbreak of Beriberi on the Empress of Asia. Jour. Royal Naval Med. Service, *3*, 195, 1917.
15. Williams, R. R., and Saleeby, N. M.: Experimental Treatment of Human Beriberi with Constituents of Rice Polishings. Philip. Jour. Sci. *B10*, 99, 1915.
16. Rowe, A. W., and Boyd, W. C.: Metabolism in Pregnancy; The Foetal Influence on the Basal Rate. Jour. Nutrition *5*, 551, 1932.
17. Dubois, E. V.: Basal Metabolism in Health and Disease. Lea and Febiger, New York, 1924.
18. Cannon, A.: Some Observations on Beriberi. Brit. Med. Jour. *2*, 852, 1929.

CHAPTER XXI

THIAMIN REQUIREMENT IN RELATION TO PROPORTION OF CARBOHYDRATE, FAT, AND PROTEIN

In the previous chapter we have considered the thiamin requirement in relation to the total food intake. The reader perhaps noted that most of the experimental diets used in such studies were high in starch or sugar. A qualification of the conclusion as to the effect of the total food intake on the thiamin requirement is, therefore, necessary. There is indeed positive evidence that the major constituents of foodstuffs are not equivalent to one another in their effect upon the thiamin requirement. Carbohydrate hastens the onset of polyneuritis in animals fed on a diet of low vitamin B_1 content; fat has a retarding influence; protein has a less pronounced effect in either direction. We shall now scan the evidence in support of these theses.

This topic has attracted attention almost from the beginning of vitamin history. In a very early experiment Funk (1) fed four groups of eight pigeons each, on 12½ grams daily per bird, using four diets as follows: Diet A contained 60 per cent of casein, diet B 60 per cent of fat, diet C 60 per cent of starch, and diet D 60 per cent of sugar. The diets were otherwise as similar as possible. Polyneuritis occurred in an average of 40 days on the high-fat diet and in 24 to 30 days on the three other diets, the difference among the latter three being hardly sufficient to be certainly significant. However, of these the most rapid onset, averaging 24 days, occurred with the high-starch diet.

Randoin, Simonet, and Lecoq have jointly and severally published a large number of experiments in which pigeons or rats were fed upon diets varying with respect to proportions of protein, fat, and carbohydrate. These papers are largely unsatisfactory, since detailed data often are not given, since the number of animals used for each experiment is small, and since there is often a serious question with regard to the freedom from thiamin of various dietary components. One of these experiments (2) deserves more detailed mention. The essential results follow:

	Diet I	*Diet II*	*Diet III*	*Diet IV*
Peptone	16%	24%	82%	59%
Butter	4%	8%	4%	8%
Lard	—	18%	—	18%
Sugar	66%	35%	—	—
Salts	4%	5%	4%	5%
Agar-agar	8%	8%	8%	8%
Filterpaper	2%	2%	2%	2%
Days survival	16–25	20–30	20–25	60–90

In this case it seems to be clear that the longer survival of pigeons on diet IV must be due in part to the proportions of the diet and not wholly to the B_1 content of any component, since each of the principal components occurs in at least one of the other diets in equal or larger amount. In the light of other supporting evidence, such as that of Evans and Lepkovsky, which we shall presently discuss, it is safe to attribute these results at least in part to lard. It is of interest, however, to note that lard had little protective action in diet II, where it was accompanied by a substantial amount of sugar. Qualitatively this confirms other evidence of the adverse effect of carbohydrates as well as of the benefits of fat. Only when the proportions of the several components were most favorable, as in diet IV, did extended survival occur. Probably traces of thiamin were present in some of the components, notably peptone.

More substantial and consistent evidence on the effect of fat began to accumulate with the work of Evans and Lepkovsky. These authors (3) showed that more thiamin is required for growth of rats on a diet of sucrose and casein than is the case when a portion of the sucrose is replaced by fat. In the presence of 50 per cent of fat, animals were found to live on a supposedly thiamin-free diet at least six months without signs of beriberi. It is very probable, however, that small amounts of thiamin were supplied by the autoclaved yeast used as a supplement to supply thermostable B factors. These authors also noted that animals partially depleted of thiamin showed a reduced tolerance for glucose. Some typical diets (4) used by them in later experiments follow:

Number of Diet	540	542	414	550	415	563	564	565	551	559	571	572	573	579
Casein	25	20	19	20	20	23	23	23	27	36	38	38	38	40
Autoclaved yeast	—	10	10	10	10	10	10	10	—	10	10	10	10	—
Sugar	75	70	—	59	—	56	56	56	59	—	—	—	—	—
Cornstarch	—	—	67	—	59	—	—	—	—	—	—	—	—	—
Salts	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Lard	—	—	—	10	10	—	—	—	10	50	—	—	—	53
Crisco	—	—	—	—	—	10	—	—	—	—	50	—	—	—
Wesson Oil	—	—	—	—	—	—	10	—	—	—	—	55	—	—
Synth. Wesson Oil	—	—	—	—	—	—	—	10	—	—	—	—	55	—

High-carbohydrate diets 542, 550, 563, and 564, used without supplement, gave substantially equal but somewhat meager growth. High-fat, low-carbohydrate diets 559, 571, and 572 gave better growth under these circumstances. When each of the diets was supplemented daily by 50 milligrams of brewers' yeast to supply a small intake of thiamin, diet 542 gave appreciably less growth than 550, 563, or 564, which contain some fat, and these in turn gave less growth than the carbohydrate-free high-fat diets 559, 571, and 572. When 200 milligrams of yeast were supplied per day, diet 542 still gave less growth than 563 or 564, but these two were no longer surpassed by 571 and 572. When 800 milligrams of yeast were given, the differences in performance among the several diets were smaller. In other words, according to this work the superiority of high-fat diets is most marked when the thiamin supply is moderately low; when the thiamin supply is liberal, the advantage of fats disappears. In the complete absence of thiamin, survival cannot occur even on high-fat diets, according to the weight of evidence that has accumulated in recent years. The present author considers that the autoclaved yeast in Evans and Lepkovsky's diets supplied a small amount of B_1 in addition to that furnished intentionally by the raw yeast addendum (5). Comparative experiments indicated that increased fat in the diet did not have any similar influence upon the amount of thermostable B factors required, a conclusion that was further confirmed by later work (6). It was also noted that dextrinized starch gave much better growth than sugar in the low-fat diets, and that hard fats were less effective in vitamin sparing, being less fully absorbed, as shown by analysis of feces. Sugar appears in many experiments of other workers. This form of carbohydrate is most prone to induce rapid onset of polyneuritis.

Later work of Evans and Lepkovsky (7) also resulted in the following comparative rating of several fats and fatty acid esters with respect to their value in preventing polyneuritis and in maintaining growth of rats on diets of low B_1 content:

Palmitin	Poor	Laurin	Positive
Myristin	Best	Caprylin	Excellent
Stearin	Negative	Ethyl palmitate	Positive
Cotton seed oil	Negative	Ethyl stearate	Negative

The above comparisons refer to experiments in which thiamin was completely absent, except as it may have been supplied by autoclaved yeast. When 50 milligrams of yeast were added, the fatty diets gave much-improved growth, and the relative rank in merit of the several fats was somewhat altered. With one gram of yeast, growth was equally good on the fat-free and fatty diets, except that the stearate diet was worse than the fat-free. The adverse effect of high-melting fat such as stearate appeared

to be due to faulty absorption, as much fat was again found in the feces. In this entire later set of experiments the fats which were used were resynthesized from purified isolated fatty acids, thus excluding any possibility of retention of traces of thiamin in the fats. The evidence of the superiority of certain glycerides over others is especially interesting. These authors showed (8) that administration of fat intraperitoneally was also effective in reducing the thiamin requirement. It was also noted that mixed glycerides of oleic and stearic acids were superior in growth-promoting action to the glyceride of either acid separately.

Evans, Lepkovsky, and Murphy (9) observed that the optimum thiamin-sparing action of fats is not attained unless the protein content and thermostable B factor supply in the diet are both high. They also extended the earlier observations as to the relative merits of a large number of natural oils and fats, including certain hydrogenated products.

The work of others, which was carried out partly during the course of Evans and Lepkovsky's studies, also added weight to the view that fats have a beneficial influence. We will first review the more important papers that are favorable to this view and later take up the smaller number which point in a somewhat different direction. B. Janowskaja (10), using carefully purified muscle protein, fibrin and ovalbumin, dextrine, rice starch, sugar, and sunflower seed oil, investigated the effect of varying the components in the diet of pigeons. With high-carbohydrate diets at an intake of 10 to 15 grams daily the birds succumbed in 13 to 28 days. With a high-protein, high-fat, carbohydrate-free diet, there was survival from 30 to 39 days on 10 grams of the ration daily and for a more variable and somewhat longer period of time on a ration of 15 grams daily. Guha (11) found a reduction of the thiamin requirement by inclusion of lard in the diet but noted no effect of palm kernel or olive oil.

Confirmation of the more general aspects of these researches on the vitamin-sparing action of fats is also found in the experiments of Westenbrink (12) on the rapidity of development of polyneuritis in pigeons fed on synthetic diets high in sugar as compared with those high in fat. Twenty birds on high-sugar diet developed polyneuritis in 19 ± 4 days; on high-fat diet in 27 ± 7 days. Salmon and Goodman (13) noted that pecan oil prevented beriberi in pigeons when given in amounts of 2.6 grams daily, and also noted briefly a general confirmation of the results of Evans and Lepkovsky regarding the effectiveness of certain synthetic fatty acid esters on rats reared on low-thiamin rations.

An extended and elaborate re-examination of the subject has since been published by Salmon and Goodman (14), with strongly confirmatory results. The following table regarding the effect of natural fats is taken from their paper:

TABLE XXI

AVERAGE GAIN IN WEIGHT AND TIME TO ONSET OF BERIBERI IN RATS RECEIVING VITAMIN B DEFICIENT DIET CONTAINING VARIOUS FATS AT A LEVEL OF 40 PER CENT *

KIND OF FAT	NUMBER OF RATS	AVERAGE TOTAL GAIN	BERIBERI INCIDENCE	AVERAGE TIME TO ONSET
		gm.	*%*	*days*
Myristin 30 / Hard lard 10	6	47	0	— †
Coconut	6	28	83	60
Cottonseed	11	6	100	51
Pecan	6	9	100	46
Hydrogenated cottonseed	24	12	95	40
Linseed	8	5	100	40
Hard lard	18	20	83	40
Soft lard	6	17	100	37
Hard lard 30 / Myristin 10	6	17	100	37
Beef	11	5	100	35
Olive	6	14	100	35
Butter	7	21	100	34

* Each rat received 250 mg. autoclaved yeast and 0.10 ml. cod liver oil per day.
† No beriberi at end of 102 days.

In these experiments 40 per cent fat was used in a diet otherwise composed as follows: Casein 22.0, salts 5.7, agar 1.0, sucrose 28.8 per cent. This was supplemented by 250 mg. autoclaved yeast and 0.10 cc. cod liver oil per rat daily. It is of interest from a dietetic standpoint that olive oil, butter, and beef fat were not very effective in retarding the onset of beriberi. Coconut, cottonseed, and pecan oils, in the order named, were the most effective of the natural fats in producing growth and in delaying the onset of beriberi as well. Lard was inferior to these in delaying beriberi, but was relatively good in promoting growth, as was also the case with butter. The data of this paper have been of great value in appraising human diets (Chapters VII and VIII). It is clear, however, that no simple expedient will provide an accurate gauge of the effect of fats in a mixed human diet. This is brought out by the marked contrast in the effects of myristin and hard lard in the proportions of 3 to 1 and 1 to 3 respectively. The effect of high fat, however, almost certainly accounts for the proved adequacy of the diet of the Eskimo from the standpoint of its thiamin content.

An extended series of synthetic esters was also studied with results consistent in general with those of Evans and Lepkovsky. The diet used was as follows: Casein 18.0, salts 4.0, agar 1.0, fatty acid as ester 23.0, sucrose to make 100 per cent. This was supplemented by 250 mg. or 500 mg. autoclaved yeast and 0.1 cc. cod liver oil and 0.2 cc. linseed oil per rat per day.

TABLE XXII

AVERAGE GAIN IN WEIGHT AND TIME TO ONSET OF BERIBERI IN RATS RECEIVING VITAMIN B DEFICIENT DIETS [1] CONTAINING VARIOUS GLYCERYL AND ETHYL ESTERS

DIET	ACID EQUIVALENT	NUMBER OF RATS	AVERAGE GAIN, 6 WEEKS [4]	AVERAGE GAIN 12 WEEKS [4]	BERIBERI INCIDENCE	AVERAGE TIME TO ONSET [4]	ESTER RETAINED
	%		gm.	gm.	%	days	%
Series A [2]							
G. caprylate	55	4	57	Discontinued	0	—	99.2
G. caprylate	36	4	51	Discontinued	0	—	—
G. caprylate	23	4	57	101	0	—	98.4
G. caprylate	5	4	47	75	0	—	—
G. caproate	23	4	46	72	0	—	99.0
G. iso-caproate	23	4	46	Discontinued	0	—	98.7
Et. caproate	23	4	42	64	0	—	—
G. heptoate	23	4	38	64	0	—	98.0
G. laurate	23	4	31	63	0	—	97.4
G. myristate	23	4	28	47	0	—	96.3
G. nonylate	23	4	23	42	0	—	98.4
G. undecylate	23	4	20	40	0	—	98.8
Et. oleate	23	4	23	34	0	—	96.9
G. propionate	23	4	19	33	0	— [5]	98.9
G. lactate	23	4	17	27	0	—	94.6
G. oleate	23	4	15	25	0	—	97.4
G. caproate	5	4	12	32	0	—	—
G. valerate	23	4	17	17	0	— [6]	98.7
Low-fat diet	—	18	7 [7]	Died	77	43	93.5
Coconut	23	8	13 [7]	Died	62	40	97.6
Et. palmitate	23	4	0 [8]	Died	75	38	92.6
G. acetate	23	4	24 [7]	Died	100	32	99.0
G. palmitate	23	4	0 [8]	Died	100	30	91.5
G. stearate	23	4	0 [8]	Died	100	17	74.4
G. butyrate	23	4	0 [8]	Died	—	20 [9]	97.6
Series B [3]							
G. caprylate	5	4	10	11	25	63	—
Low-fat diet	—	14	0 [8]	Died	100	25	—

[1] Each rat received 0.10 ml. cod liver oil and 0.20 ml. linseed oil per day.
[2] Each rat in series A received 500 mg. autoclaved yeast per day.
[3] Each rat in series B received 250 mg. autoclaved yeast per day.
[4] All rats received the low-fat diet for a 2-week depletion period; the time of experiment was reckoned from the end of this period.
[5] One rat died three days after starting on glyceryl propionate.
[6] One rat died three days after starting on glyceryl valerate.
[7] Average gain to maximum weight.
[8] These rats lost weight from start of ester-containing diets.
[9] Average length of life; symptoms not typical of beriberi.

Glycerol caprylate, caproate, isocaproate, heptoate, laurate, myristate, nonylate, undecylate, propionate, lactate, oleate and valerate, also ethyl caproate and oleate, were able to prevent completely the occurrence of beriberi when 500 mg. daily of autoclaved yeast were given each rat. The efficiency of fatty acids appears to depend on the length of the carbon chain, a chain of eight atoms representing the approximate optimum. Glyceryl acetate, palmitate, and stearate were not effective in preventing beriberi under precisely the same conditions. In view of this fact

one cannot ascribe the protection wholly to residual vitamin B_1 in the autoclaved yeast, although the authors of the paper believe that significant traces of thiamin were present in it. Striking cures of polyneuritis in rats were obtained by feeding a diet containing 23 per cent of synthetic glyceryl caprylate or caproate. The authors do not believe a high intake of protein or of thermostable B factors is a requisite to securing the thiamin-sparing action of fats, although a high intake of autoclaved yeast makes the benefits of fats more conspicuous. Fats do not completely obviate the necessity for B_1, but ample B_1 obliterates the advantage of the fats.

While these results leave no doubt in our minds as to the genuineness of the beneficial action of fats, we need not subscribe to the idea that fats are effective because they conserve the store of thiamin in the body. Indeed the cure of polyneuritis by the aid of fats indicates clearly that such is not the case. This meets the principal objection of those who have hesitated to accept the particular interpretation which Evans and Lepkovsky have given to their results, namely, that fats delay the depletion of thiamin in the tissues and are effective for that reason (15). This view is shared by Guerrant and Dutcher (16). Gregory and Drummond (17) obtained results which have been cited in opposition to the idea that fats are beneficial, but a careful reading of the paper does not justify this construction. Improvement in growth was noted in rats on diets low in thiamin, but the authors argued that the action of fats is not truly a thiamin-sparing action, since fats afford no benefit if thiamin is entirely absent. This is really in good accord with the experimental results of others. The difference lies only in the interpretation.

To settle the question of interpretation, Kemmerer and Steenbock (18) assayed the tissues of rats, chickens, and swine fed on rations deficient in thiamin and high and low in fat respectively, and compared them as to thiamin content with those of normal animals. They found that the thiamin content of the tissues of animals fed on the various rations depended on the thiamin content of the rations and not on the fat content. The tissues of the animals fed on the low-thiamin diets were low regardless of whether the fat content was high or low. However, in accordance with the work of various others above cited there was less weight loss on high-fat diets than on low ones when the thiamin supply was scanty.

Westenbrink (19) is fully in agreement as to the beneficial influence of fat but regards the phenomenon as due to lessened intake of carbohydrate rather than increased fat. By feeding pigeons on equal amounts of carbohydrate daily supplemented by different amounts of fat he could not appreciably delay symptoms. He regards the symptoms as a chronic carbohydrate

poisoning. He also argues that the beneficial action of fat is not a vitamin-sparing one on the ground that depletion of thiamin content of tissues takes place long before the symptoms appear.

Evans and Lepkovsky (15) disagree with the findings (Kemmerer and Steenbock) as applied to young rats. They assayed the tissues of young rats fed for forty days on a low-thiamin diet, high in fat in one case, low in another. It was found that the tissues of the rats on the high and low fat diets were much alike in thiamin content, weight for weight, except in the case of the liver. In that instance the thiamin content was higher in the animals fed on the high-fat diet. But as the bodies of the animals grown on the high-fat diet were twice as heavy as those on the low-fat diet, the total thiamin content of their bodies must have been substantially greater. This view is supported by details of comparative weight of musculature, livers, and brains and the thiamin assays of each. As the rats whose tissues were assayed received equal amounts of thiamin daily in the form of an extract of rice bran, regardless of whether the diet contained little or much fat and regardless of how much food they ate, it appears that the high-fat animals conserved their supply of thiamin better. This is not, however, quite as conclusive as it seems. If the tissues of the high-fat animals contained more fat, the higher fat content, *ceteris paribus,* would be reflected in better growth of the animals used for testing the thiamin content of the tissues and would accordingly tend to result in too high values for thiamin content. Evans and Lepkovsky, however, are disposed to account for the contrast in the two sets of results as due to the use of adult animals by Kemmerer and Steenbock, the thiamin supply being in their view insufficient for adults even on high-fat diets, although sufficient for the young.

The relation of fat to the phenomenon of thiamin synthesis in the gastro-intestinal tract has been studied by Whipple and Church (20). They found that the feces of rats on a thiamin-deficient, high-fat diet contained thiamin. When the fat was removed from the diet, the thiamin disappeared from the feces. The above results were observed with a diet containing sucrose. With cornstarch as substitute for sucrose only a small thiamin content of the feces was noted. This was attributed to a little fat in the starch. Fat was found to relieve the symptoms of beriberi in rats which had developed the disease on a fat-free diet but was not adequate to protect them indefinitely.

The intestinal flora of rats can synthesize thiamin. This takes place principally in the caecum (Guerrant, Dutcher, and Tomey (21)). When such organisms are established in a rat colony, they spread widely through coprophagy and have occasioned much trouble in vitamin B_1 testing. This complication may well have added serious difficulties to the interpretation of

Whipple and Church's results on the thiamin-sparing action of fat. However, there is much reason to doubt that this explanation will hold for the experiments of others, for example, those of Evans and Lepkovsky and of Salmon and Goodman. These authors were quite aware of the phenomenon of refection and gave it careful consideration. Refection is usually rather easily recognized by the large, light-colored feces which result from it. Guerrant, Dutcher, and Brown (22) have studied the phenomenon reported by Whipple and Church and conclude that high fat in the form of cottonseed oil does not favor synthesis in the intestines. A similar conclusion has been reached by Kelly and Parsons (23) as to the unimportance of fat as a factor in refection.

A few investigators have failed to substantiate the beneficial effects of fats. Yonechi (24) was unable to perceive any favorable effect of added cottonseed oil as a supplement to a polished rice diet for pigeons, judging either from weight loss or the time of onset of polyneuritis. In rats also, beneficial effects on growth and occurrence of beriberi were almost entirely absent. Yonechi concluded that fats and oils hasten the onset of polyneuritis, the more so the greater the vitamin A content of the diet. A large number of variables was introduced in the experiments, and the prosecution of the investigation does not appear very systematic. The most probable explanation in the case of the rat experiments is that the supply of autoclaved yeast was quite low. Sure and Buchanan (25) found no thiamin-sparing action of high-fat diets even if vitamin B_2 and protein are present in excess. This finding, which appears to be at variance with those of Evans and Lepkovsky, has not been supported as yet with sufficiently detailed data to permit a final judgment. E. W. McHenry (private communication) has called attention to the fact that most of the diets used in the various experiments on the thiamin-sparing action of fats have involved autoclaved yeast, an addendum which supplies liberal amounts of choline. McHenry considers the presence of choline essential to the sparing action (26). It is noteworthy that the diet which is described in detail in the above-cited paper of Sure and Buchanan makes use of autoclaved beef rather than autoclaved yeast. Reference is made, however, in the same paper to other experiments which are not reported in detail, which involved diets containing, in certain cases, unspecified amounts of yeast, or yeast products, and which likewise were said to reveal no sparing action of fat. The cited experiments of Yonechi also lend color to McHenry's view, as the choline supply must have been relatively low in Yonechi's diets. We are inclined to believe that McHenry's explanation is correct in this particular.

McHenry also expresses a doubt whether the sparing action

of fats in birds is genuine because they have a much lesser capacity for absorbing fats than do most mammals. The various reports of cure of polyneuritis in pigeons by administration of fats would indicate that they can absorb significant quantities of fat. On the other hand, pigeons are notoriously subject to spontaneous or anomalous cures of polyneuritis (see page 208) and it seems best to reserve final judgment on this matter.

It will be well to call the reader's attention at this point to the fact that small amounts of certain unsaturated fatty acids containing more than one double bond are now regarded as one of the essentials for proper nutrition of higher animals (Evans and Lepkovsky (27, 28)). The required amounts are very minute, so that this need can be distinguished readily from the influence of fats on thiamin requirement.

Approaching the matter of the proportion of the components of the diet from a wholly different viewpoint, Abderhalden and Wertheimer (29) noted that at the onset of paralytic symptoms in pigeons there was uniformly an increase of liver glycogen above normal. Administration of B_1 not only dissipated the neuritic symptoms but also decreased the glycogen. The appearance of high glycogen occurred in avitaminosis even when there was no carbohydrate in the diet but was limited to those cases in which typical paralytic symptoms appeared. Mere inanition results in low liver glycogen. Indeed the paralytic symptoms appear to be thought of as due in a sense to high liver glycogen and consequent liability of spillage of sugar into the blood. On diets consisting of 3 parts casein and 7 parts olive oil, polyneuritis occurred regularly and the glycogen contents of the tissues were high. Even with diets containing only 1 part casein to 16 parts olive oil occasional polyneuritis occurred, and when it did, was also associated with high glycogen, *e.g.*, 2.8–3.0 per cent in the liver. If yeast was added to these casein-fat diets, polyneuritis, of course, did not occur and it was also found that the glycogen values were kept at normal levels, *e.g.*, 0.77 per cent in the liver. By contrast, on a diet without yeast, containing 10 parts casein to 5 parts fatty acid, 6.3 per cent of liver glycogen was encountered upon occurrence of polyneuritis. High-fat diets were found to delay polyneuritis up to eighty to one hundred days; high carbohydrate or high protein (carbohydrate builder) hastened it. Feeding pigeons on fatty acids alone never produced polyneuritis but only a progressive state of exhaustion which was not remedied by yeast. Feeding on pure olive oil produced two cases of polyneuritis out of fifteen birds, due, in the authors' opinion, to the metabolism of the glycerine of the fat. Those which escaped polyneuritis showed low glycogen values. One of the two polyneuritic birds, however, was found to have 3.6 per cent glycogen in the liver; the other, after repeated cramps,

0.62 per cent. These authors emphasize that there is no relation between weight loss and the incidence of polyneuritis; attacks may occur when the weight curve is rising. They believe that fats do not replace or spare vitamin but only tend to prevent the appearance of symptoms by displacing carbohydrate in the diet or by repressing the formation of carbohydrate from protein in the body by lessening the protein intake. The work of these authors, however, is not altogether convincing, as the diets were extremely artificial and highly deficient in many ways. Also the individual variations of the animals were large and the authors exercised free judgment in classifying the birds according to the symptoms exhibited. Much depends on the accuracy of their observations and judgments as to the relative predominance of inanition and polyneuritis in particular cases—judgments which the reader has little opportunity to verify.

However, the viewpoint of Abderhalden and Wertheimer is logical, as it is well known that proteins function in lieu of carbohydrates in metabolism when the supply of the latter in the diet is low. Their view is strikingly borne out in the analysis of human diets which has been presented in Chapters VII and VIII. For such diets, taken as a whole, the thiamin-non-fat-calorie ratio was found to be a much better index of adequacy than the thiamin-carbohydrate ratio or the thiamin-total-calorie ratio.

We will now do well to examine other evidence as to thiamin requirement in relation to protein intake. Funk and Collazo (30) brought pigeons into B_1 balance on a diet containing 12½ per cent of protein. Then upon transferring them to a diet of 25, 50, or 75 per cent protein, weight gains occurred more or less proportionately to the protein. Decline occurred when the protein was decreased and the carbohydrate increased. The validity of such results, of course, depends upon the strict absence of thiamin in the protein. This is difficult to assure completely. Moreover, the experiment is not shown to relate specifically to the thiamin requirement but merely to the state of nutrition of the birds.

Reader and Drummond (31) concluded that low-protein diets may be improved for the growth of rats by increasing the proportion of yeast extract. No definite conclusion can be drawn from this as to the relationship between thiamin and protein needs, as these experiments were performed before vitamin was differentiated into thermolabile and thermostable factors.

Guha (11) reported that the B_1 requirement of pigeons is independent of the carbohydrate-protein ratio, implying that these two components of foods are equivalent in this respect. Mitchell (32) reported indications that the thiamin requirement of rats is reduced by high protein in the diet.

Hogan and Pilcher (33) compared the growth of rats under the following set of conditions:

(*a*) Protein low, thiamin low.
(*b*) Protein low, thiamin liberal.
(*c*) Protein high, thiamin liberal.
(*d*) Protein high, thiamin excess.

Rats receiving diet (*b*) consumed more food than those on diet (*a*) and accordingly grew faster. When the protein was raised as in diet (*c*), food consumption was not increased but growth was. In the case of diet (*d*) both food consumption and growth were still further enhanced.

In further experiments, feeding was regulated to a constant level in all cases, whereupon better rates of growth could be noted on the higher protein diets, but the higher thiamin diets did not give any more enhancement of growth than could be ascribed to the calorific value of the supplement. The thiamin required for a given rate of growth did not appear to vary with the protein content of the diet, but when both thiamin and protein were deficient, better growth was obtained by adding either one to the food.

Summary

1. Decreased carbohydrate intake has convincingly been shown to delay the onset of polyneuritis; abundant carbohydrate increases the thiamin requirement.

2. The substitution of fat for carbohydrate lessens the thiamin requirement, delays onset of polyneuritis, and permits growth on low thiamin intakes.

3. Fats vary in efficacy in this respect, those of intermediate length of fatty acid chain being markedly superior.

4. The proportion of protein, so long as the amount is sufficient for proper nutrition, does not appear to influence the thiamin requirement conspicuously in animal experiments.

5. Protein appears in some experiments to function as a carbohydrate former. See p. 101 for its effect in human diets.

6. The presence of adequate choline is probably necessary to secure the sparing action of fat on the thiamin requirement.

BIBLIOGRAPHY

1. Funk, C.: Die Rolle der Vitamine beim Kohlenhydrat-Stoffwechsel. Zeit. physiol. Chem. *89*, 378, 1914.
2. Lecoq, R.: Le Rôle des Vitamines B et de l'Equilibre Alimentaire dans l'Utilization des Protides. Compt. Rend. *194*, 1267, 1932.
3. Evans, H. M., and Lepkovsky, S.: On Some Relations of Vitamin B to Fat and Carbohydrate Metabolism. Amer. Jour. Physiol. *90*, 340, 1929.

4. Evans, H. M., and Lepkovsky, S.: Sparing Action of Fat on the Antineuritic Vitamin B. Jour. Biol. Chem. *83*, 269, 1929.
5. Williams, R. R., Waterman, R. E. and Gurin, S.: Effect of *p*H Control in the Autoclaving of Yeast with Respect to the Vitamin B Factors. Jour. Biol. Chem. *83*, 321, 1929.
6. Evans, H. M., and Lepkovsky, S.: Sparing Action of Fat on Vitamin G. Jour. Biol. Chem. *107*, 443, 1934.
7. Evans, H. M., and Lepkovsky, S.: The Sparing Action of Fat on Vitamin B. The Rôle Played by Glycerides of Single Fatty Acids. Jour. Biol. Chem. *96*, 179, 1932.
8. Evans, H. M., and Lepkovsky, S.: The Sparing Action of Fat on Vitamin B. Is It Necessary for Fat to Interact with Vitamin B in the Alimentary Canal to Exert Its Sparing Effect? Jour. Biol. Chem. *99*, 235, 1932.
9. Evans, H. M., Lepkovsky, S., and Murphy, E. A.: The Sparing Action of Fat on Vitamin B. Influence of the Level of Protein and Vitamin G. Jour. Biol. Chem. *107*, 429, 1934.
10. Janowskaja, B.: Zur Frage nach dem Einfluss der Kohlenhydrat auf die Entwicklung der polyneuritischen Avitaminose bei Tauben. Biochem. Zeit. *238*, 125, 1931.
11. Guha, B. C.: Physiological Function of Vitamin B_1. Biochem. Jour. *25*, 1367, 1931.
12. Westenbrink, H. G. K.: Über den Einfluss der Menge und der Zusammenstellung der Nahrung auf die Folgen von Vitamin-B_1 Mangel. Arch. Neerl. Physiol. *19*, 94, 1934.
13. Salmon, W. D., and Goodman, J. G.: Efficacy of Fats in Decreasing the Vitamin B Requirement of Pigeons and Rats. Ala. Agri. Exp. Sta. Annual Rept. *42*, 42, 1931; *44*, 22, 1933; *45*, 21, 1934.
14. Salmon, W. D., and Goodman, J. G.: Alleviation of Vitamin B Deficiency in the Rat by Certain Natural Fats and Synthetic Esters. J. Nutrition *13*, 477, 1937.
15. Evans, H. M., and Lepkovsky, S.: Sparing Action of Fat on Vitamin B. On the Loss of Vitamin B from the Rat's Tissues. Jour. Biol. Chem. *108*, 439, 1935.
16. Guerrant, N. B., and Dutcher, R. A.: Some Effects of the Composition of the Diet on the Vitamin B and the Vitamin G Requirement of the Growing Rat. J. Nutrition *8*, 397, 1934.
17. Gregory, E., and Drummond, J. C.: A Study of Fat Metabolism with Special Reference to Nutrition on Diets Devoid of Fat. Zeit. f. Vitaminforsch. *1*, 257, 1932.
18. Kemmerer, A. R., and Steenbock, H.: Study of the Sparing Action of Fats on the Vitamin B Content of Animal Tissues. Jour. Biol. Chem. *103*, 353, 1933.
19. Westenbrink, H. G. K.: Über die physiologische Wirkung des Vitamins B_1. Arch. Neerl. Physiol. *20*, 481, 1935.
20. Whipple, D. V., and Church, C. E.: Relation of Vitamin B (B_1) to Fat Metabolism. The Rôle of Fat in the Refection Phenomenon. Jour. Biol. Chem. *109*, 98, 1935.

21. Guerrant, N. B., Dutcher, R. A., and Tomey, L. F.: Effect of the Type of Carbohydrate on the Synthesis of the B Vitamins in the Digestive Tract of the Rat. Jour. Biol. Chem. *110,* 233, 1935.
22. Guerrant, N. B., Dutcher, R. A., and Brown, R. A.: Further Studies Concerning the Formation of the B-vitamins in the Digestive Tract of the Rat. J. Nutrition *13,* 203, 1937.
23. Kelly, E., and Parsons, H. T.: Quantitative Studies on Refection in the Rat. J. Nutrition *13,* 453, 1937.
24. Yonechi, S.: The Biological (Synergistic) Interrelationships between Fat, Vitamin A and Vitamin B. Tohoku J. Exper. Med. *24,* 249, 1934.
25. Sure, B., and Buchanan, K. S.: Avitaminosis. XVII. Influence of High Fat-containing Diets on Vitamin B_1 Requirements. Proc. Soc. Exp. Biol. Med. *33,* 75, 1935.
26. McHenry, E. W.: An Effect of Choline on the Vitamin B_1-sparing Action of Fats. Biochem. J. *31,* 1616, 1937.
27. Evans, H. M., and Lepkovsky, S. Vital Need of the Body for Certain Unsaturated Fatty Acids, I. Jour. Biol. Chem. *96,* 143, 1932.
28. Evans, H. M., and Lepkovsky, S.: Vital Need of the Body for Certain Unsaturated Fatty Acids, II. Jour. Biol. Chem. *96,* 157, 1932.
29. Abderhalden, E., and Wertheimer, E.: Beziehungen des Vitamin-B-Komplexes (insbesondere des Vitamin B_1) zum Kohlenhydrathaushalt. Arch. f. d. ges. Physiol. *233,* 395, 1933.
30. Funk, C., and Collazo, J. A.: Die Zusammensetzung der Nahrung unter der Vitaminbedarf. Chem. Zelle Gewebe *12,* 195, 1925.
31. Reader, V., and Drummond, J. C.: Relation between Vitamin B and Protein in the Diet of Growing Rats. Biochem. J. *20,* 1256, 1926.
32. Mitchell, H. H.: Application of Paired Feeding Method to Quantitative Estimation of Relative Vitamin B Contents of Foods and Artificial Concentrates. Amer. Jour. Physiol. *104,* 594, 1933.
33. Hogan, A. G., and Pilcher, R. W.: Effects of Variations in the Amounts of Vitamin B and Protein in the Ration. Univ. Missouri Agri. Exper. Sta. Research Bull. 195, 1933.

CHAPTER XXII

THIAMIN REQUIREMENT IN RELATION TO SPECIAL CIRCUMSTANCES

In the foregoing discussion of thiamin requirement we have confined ourselves to a consideration of the requirement necessary to protect against polyneuritis and death. One might well predict from *a priori* considerations that the minimum necessary for such protection would prove insufficient for the complete well-being of animals and for the performance of all the natural functions. Such indeed appears to be the case.

In Chapter XVII extended reference has been made to the influence of the quantity of thiamin administered upon the weight maintenance of pigeons and upon the growth rate of rats. In this connection data have been presented indicating the effect on young rats of a range of vitamin intakes varying from 1 to 10 γ daily. A wide range of growth rates was observed with addenda of this order of magnitude. In the experiments of Waterman and Ammerman (1) the daily increment of thiamin given to rats on the Chase-Sherman diet was extended progressively up to 160 γ. As shown in Figure 12, every increment of thiamin supply resulted in some further increase in the growth rate, though apparently under a law of diminishing return at the higher levels. The weight curves of some of these animals receiving 160 γ of thiamin per day for a period of 1½ years are shown in Figure 13. The experiments were continued through a second generation of offspring without encountering any indication that the somewhat supernormal growth rates observed would not persist indefinitely in a succession of generations or that the effect of high thiamin intake was either lessened or intensified in subsequent generations.

When we performed the experiment of giving graduated high doses of thiamin in a similar fashion to rats on our stock breeding diet consisting of one third whole milk and two thirds whole wheat, we encountered some surprising results. The thiamin content of this diet was determined by extracting it with acidulated 80 per cent alcohol and feeding the extract to polyneuritic rats. The thiamin content was found to be about 4 γ per gram. Thus the rats received about 40 γ of thiamin daily from

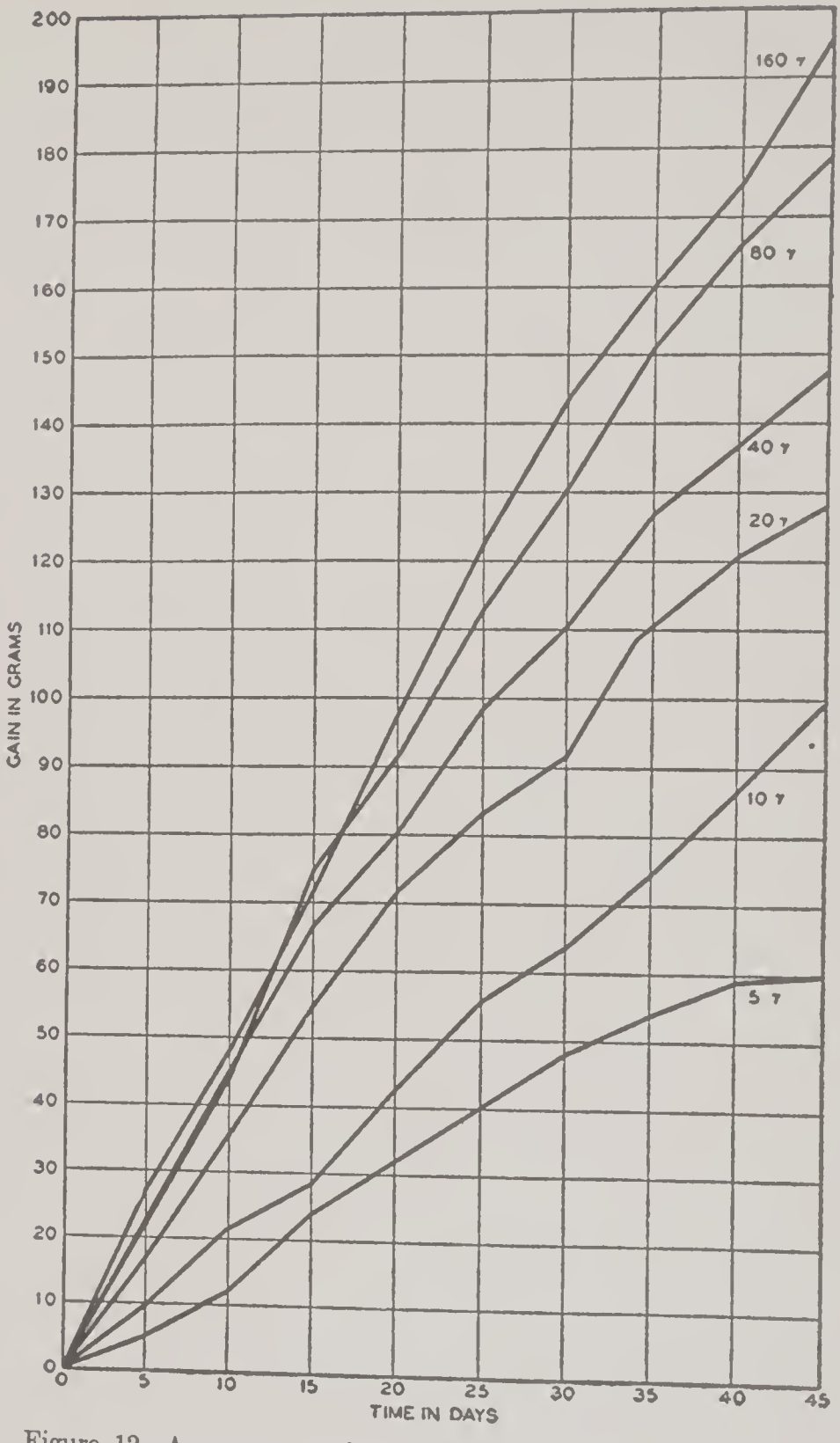


Figure 12. Average growth curves of male rats receiving large amounts of crystalline vitamin B_1. The addenda were given every 5 days. The chart shows the intakes of vitamin calculated on a daily basis. The addenda were started after 28-day-old rats had been depleted 15 to 17 days on a B_1 free diet. Courtesy of *Journal of Nutrition.*

the diet. Daily administration of additional quantities of crystalline natural vitamin to these rats led to scarcely any measurable enhancement of the growth rate (Fig. 13), although the intake derived from the diet was far below the amount which had proved stimulative to weight increase of rats on the Chase-Sherman diet. It is indeed remarkable that the stock diet composed of natural foods should result in definite limitation of growth and at the same time induce excellent performance with respect to other functions. The stock diet in question is one which has been used successfully in Professor Sherman's laboratory as well as

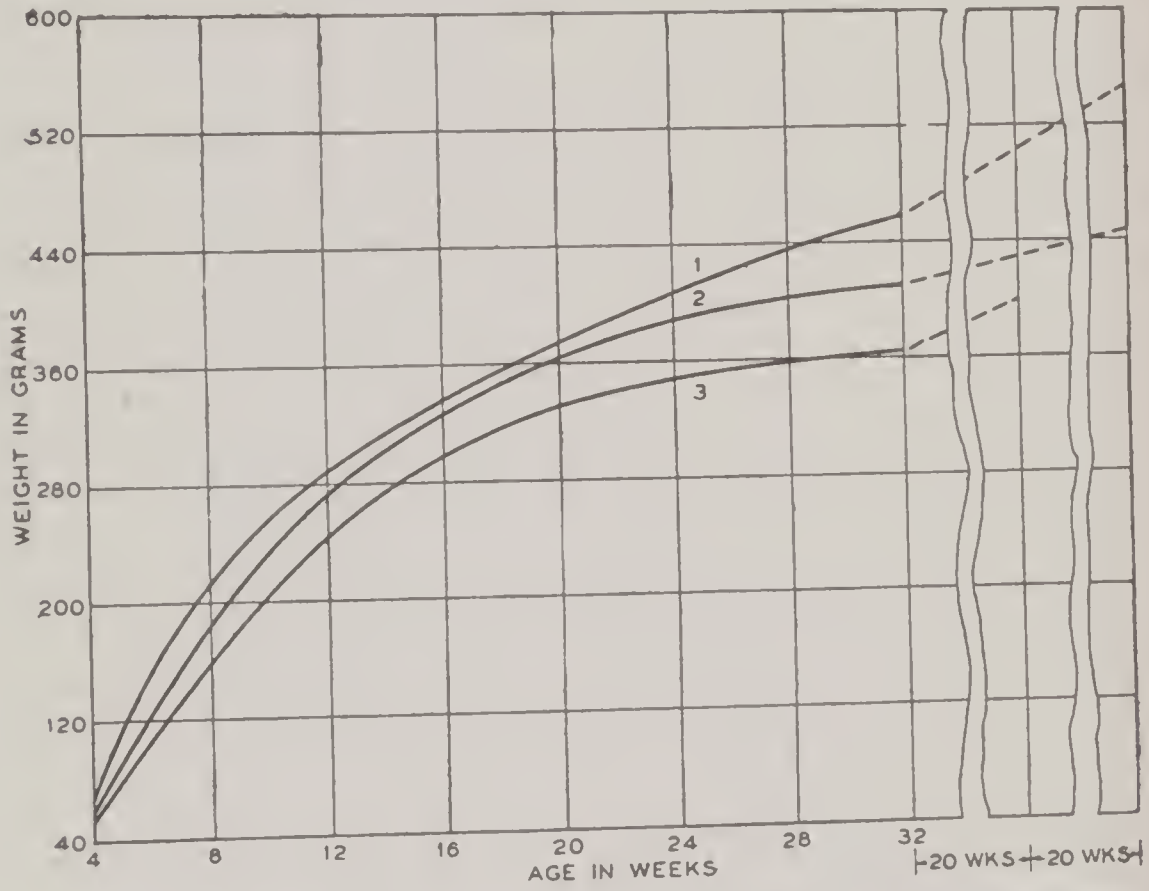


Figure 13. Effect of diet on stimulation of growth of male rats (litter mates) by large amounts of thiamin chloride.

1. Diet 515 + 160 thiamin chloride daily.
2. Diet 515 + 40 thiamin chloride daily.
3. Diet 12 M + 0.0, 50, 250 and 1000 γ thiamin chloride, respectively, daily.

The influence of the graded amounts of thiamin chloride was less than that of the individual variation. Curve 3 is typical of the whole group.

Diet 515

	Per Cent
Butter fat	8
Casein	18
Cornstarch	53
Salt Mixture	4
Cod Liver Oil	2
Autoclaved Yeast	15
Wheat oil in addition	

Diet 13 M

Whole wheat—66 parts
Whole dried milk—33 parts
Lean meat in addition every other day.

in the authors' laboratory for many years in breeding thousands of rats for experimental purposes.

In this connection it is interesting to note that Williams (2) demonstrated that pigeons could not in general survive on an exclusive diet of rice meal, bran, or polish fed *ad libitum,* although suitable addenda of any one of these products to polished rice produced the normal result of protection from polyneuritis, maintenance of weight, and a healthy condition. Several of the birds which died on the exclusive diets of rice offal exhibited nerve degeneration similar to that of beriberi and three showed head retraction or spasticity characteristic of that condition. Attention was called at that time to the previous observation of McCollum, Simmonds, and Pitz (3), who recorded evidence of the presence of a "toxic" substance in wheat.

According to McHenry (4–6), in the absence of choline in the diet, fat is deposited in the livers of rats, and this deposit is increased by the administration of further amounts of thiamin. On the other hand, administration of choline to rats on a thiamin-adequate diet promotes transport of fat from the liver to the depots and thus prevents its accumulation in the liver. Further, McHenry finds that choline supplements the action of thiamin in promoting the growth or weight maintenance of rats. On a diet low in choline as well as in thiamin, failure of growth in about two weeks was observed in McHenry's experiments. Additions of choline or thiamin did not bring about a continuation of the growth, but if both substances were added, the weights proceeded to rise. McHenry notes that choline-deficient rats display symptoms very similar to those ascribed to a B_4 deficiency. The complementary action of choline and thiamin on growth appeared less marked in older rats than in young ones. On low-choline diets thiamin caused increased fat formation in the liver even when the rats were fed on fat-free diets. On fatty diets low in choline the fat deposition rose still further in proportion to the fat content of the food. These facts obtained even when the rats receiving thiamin and the controls were kept at isocaloric intakes. When they were allowed to eat *ad libitum,* the body weight differences became very substantial.

McHenry's experiments are as yet not quite satisfactory as regards the supplementary action, as the diets used are not clearly adequate in respect to certain B factors other than thiamin. This deficiency is difficult to make good without introducing new uncertainties as to the choline intake. However, the evidence that there is an interrelationship between thiamin and choline which, in some respects, partakes of the nature of an antagonistic action and in other respects of a complementary action makes the subject attractive for further exploration. It seems quite possible that the choline-thiamin ratio of a diet may be quite important.

Perhaps, since the Toronto workers have found that betaine and other bases have an effect essentially similar to that of choline, a category of bases must be considered. The chemical resemblances of choline and thiamin are of possible interest in this connection, since both are quaternary bases and also derivatives of ethyl alcohol.

It is quite possible that the idea of a subsidiary B_4 deficiency or other deficiency in the Ammerman-Waterman experiments may also have to be invoked to secure a complete explanation. The question of whether so-called B_4 deficiency is a choline deficiency must likewise be left for future determination. Elvehjem, who has studied B_4 extensively, feels that choline is not identical with B_4 (7).

The explanation of the Ammerman-Waterman phenomenon as observed in the case of the Chase-Sherman diet, which may seem most acceptable to many readers, is that the increasing growth rates, with increasing amounts of vitamin, is due not to larger amounts of thiamin supplied but to contamination of the crystals with some other essential. This possibility was mentioned by Ammerman and Waterman in their original paper and is the one which is preferred by Elvehjem and Arnold (8). According to this view the crystalline thiamin was contaminated with a small amount of a second factor, perhaps B_4. The latter factor being present only in traces, it was necessary to add large amounts of the crystals before a sufficiency of the second factor was attained. If this is the true explanation, the Ammerman-Waterman phenomenon must be regarded as analogous to the Cowgill phenomenon which is discussed in Chapter XXIII. In the experiments of Cowgill with rats the thermostable B factors were supplied only as they occurred in the Harris yeast powder used as a source of thiamin supply. In these experiments we believe that the thermostable elements constituted the limiting factor, although Cowgill construed the results to mean that the thiamin requirement rose in proportion to a higher power of the weight attained by the animals.

We have little doubt that the Chase-Sherman diet lacks some thermolabile element other than thiamin, and it is wholly probable that this thermolabile element, whatever it is, is supplied by our "stock diet," upon which animals did not respond with increased growth when higher amounts of crystalline thiamin were supplied. These facts would appear to give strong color to the explanation favored by Elvehjem and Arnold. However, there are two facts against such an explanation: First, that the animals on the stock diet with large amounts of thiamin grew less rapidly and to lower ultimate weight levels than those on the Chase-Sherman diet with corresponding amounts of thiamin (Fig. 13). This indicates an inhibiting substance in the former

diet. The second fact against this explanation is that synthetic thiamin appears to produce the Ammerman-Waterman phenomenon in marked degree, although the range of larger doses which have as yet been tried extends only as high as 50 milligrams per day. The experiments must be repeated with synthetic thiamin and extended through a wider dosage range before a final conclusion can be reached. Perhaps the results merely indicate that excess amounts of thiamin in diets are able in large degree to compensate for certain other deficiencies.

The results of Leong (9) (see page 251) tend to confirm the observations of Ammerman and Waterman that the rat can, at least under certain circumstances, utilize with advantage amounts of thiamin far in excess of usual intakes.

In the Ammerman-Waterman experiments with rats on the Chase-Sherman diet, it is unfortunate in the light of other observations, which are presently to be discussed, that no systematic histological examination of the tissues of the various organs was made. Autopsy by dissection, however, revealed nothing abnormal or especially suspicious. Large livers were recorded in some instances, but it is doubtful whether the condition observed should be regarded as abnormal. A careful pathological examination was made of the organs of the animals which were maintained throughout life on the stock diet with addenda of thiamin as high as 1 milligram daily. These animals showed no pathological condition in any organ. The hearts showed a slight tendency to be enlarged in comparison with normals, but it is as reasonable to regard this as supernormal as to view it as pathological. In this connection it is of great interest to note that the heart rate of rats is markedly dependent on the thiamin intake. It is this fact which makes possible the use of the bradycardia test in this connection (see Chapter XVII). According to Sampson (10), the heart rate of rats on the Chase-Sherman diet with 5 γ daily of synthetic thiamin stabilizes at maturity at about 400, with 20 γ at 480 and with 50 γ at 500.

Rigler (11) attributed to crystalline thiamin, as isolated by Jansen and Donath, the property of a heart automatine, *i.e.*, a substance capable of maintaining the beating of the heart even *in vitro*. Zwaardemaker (12, 13) repeated the experiments and found the automatine property present in the thiamin only after subjection to radioactive rays. Wenckebach (14), commenting on the significance of automatine action in relation to the heart condition in beriberi, objects to the use of the word automatine in referring to a substance which restores normal heart action. It seems surprising that the above interesting observations have not been pursued further.

We must return once more to the interesting experiments of Ammerman and Waterman with rats on the Chase-Sherman diet.

These rats showed a defect in performance with respect to reproduction, which, however, had nothing to do with the level of thiamin intake, being as equally marked at low levels as at high ones. It is evidently characteristic of the basal diet. Although the litters were normal as to number, and the individual young were extremely well nourished and apparently somewhat above normal in size, there was a high mortality during the nursing period in the case of the litters born in the latter part of the life of the first generation. It was also conspicuous that the mothers exhibited little maternal solicitude for the young and in some instances the young were eaten. In the second generation there was a marked tendency to infrequent litters; high infant mortality began with the earliest litters and maternal indifference was maintained or accentuated, so that no young of the third generation survived the nursing period. This occurred in spite of the fact that the young of this generation were also unusually large and well nourished at birth. The cause of these defects in reproductive functioning is obscure. Vitamin E was supplied in the form of wheat oil, and manganese was included in the salt mixture. However, in the case of the stock diet reproduction was perfectly normal even when the stock diet was supplemented by 80 to 1000 γ of the vitamin daily. This continued through three generations kept at these high levels of thiamin intake. It is thus perfectly clear that the impairment of reproductive function on the Chase-Sherman diet with large thiamin supplements is not at all due to excessive thiamin but to the nature of the diet and probably to a deficiency in it.*

Perla (15), however, reports some results which he construes in a manner quite at variance with the foregoing conclusion. The diet used in his experiments was an extremely varied one. It included 25 parts rolled oats, 25 parts fine meat and bone, 1½ parts salt, 16 parts dried skim milk with addenda of cod liver oil, wheat germ, brewers' yeast, fresh greens, and fresh milk. Growth on this diet was good, and reproduction and rearing of young were satisfactory. One group of eight females was fed this diet with a further addendum of Mead Johnson's brewers' yeast equivalent to 50 I.U. of B_1 per day. A second group of six received a vitamin B_1 concentrate (fuller's earth adsorbate) equivalent to 50 I.U. per day. This was subsequently replaced by crystalline thiamin in equivalent amount injected subcutaneously. The experiment was continued for several generations. The growth of the animals fed the excess of thiamin was not greater than that of the controls. The females bred more regularly than the controls, but a large proportion of their litters

* Absence of Vitamins L_1 and L_2 as very recently postulated by Nakahara, Inukai and Ugami (Science *87*, 372, 1938) may well be partly or completely responsible.

was born dead, or the young were early neglected by the mothers and died or were eaten. Failure of lactation was also noted. However, a substantial number of young were reared by all groups. The experiments are somewhat unsatisfactory on account of the changes of regimen during their course. The disturbance of very young litters for purposes of daily injections seems a questionable practice which might well lead to the abandonment of the young by the mothers. The precaution observed by Ammerman and Waterman of injecting the controls with saline to parallel the thiamin injections given the young of the experimental group would have been wise. Perla's results appear at variance with those of others cited below. In any case, since the thiamin intakes were higher than can be reached by the use of normal foodstuffs, the results have little immediate practical significance.

In the light of the foregoing experiments and discussion, it is evident that variation in other minor constituents of the diet may profoundly affect the apparent B_1 requirement. It is also evident that the character of the diet may determine whether or not the fundamental functions of reproduction can be properly performed and what weight levels may be attained. Further, as was pointed out in the previous chapter, the proportion of carbohydrate and fat in the diet very greatly influences the body's need for thiamin in order to avoid the incidence of outright polyneuritis. One cannot then speak of thiamin requirement as an absolute quantity. The quantity required is relative to many factors, only a few of which we can definitely discern at present.

This viewpoint is of the utmost importance in considering the therapeutic use of thiamin as well as dietary hygiene in connection with it. At first it appeared inexplicable that persons suffering from neuritis, diabetes, gout, epilepsy, or even general malaise, etc., should report benefit from a liberal supply of this vitamin, especially in cases in which there was no evidence that the dietary intake of it was unusually low. If, however, the thiamin requirement is relative to many other factors, it is clear that what is sufficient for one individual may be insufficient for another without a cause which can be assigned at present. It is common experience that considerable individual variations exist in experimental animals. In rats our experience is that occasional animals need only half as much vitamin as is usually required to prevent polyneuritis; occasionally others require twice the usual amount. One of the authors kept a domestic fowl for nine months on polished rice without the development of polyneuritis, extreme inanition, or weakness, although it was confined to the same cage and fed upon the same diet with birds that were regularly succumbing. A few less conspicuous instances were encountered among pigeons. Possibly some peculiar form of re-

fection was present, but if so, it was not communicated to cage mates.

Disregarding such exceptional cases for the moment, it is still obvious that the average thiamin requirement of a species can only be stated with reference to certain norms of performance which must be defined with the greatest possible exactness. Such considerations as these as applied to medical practice are discussed in Chapter IV, page 70 *et seq.*

Thiamin in Relation to Appetite

An early and important reflection of thiamin deficiency is loss of appetite. Such loss of appetite may result alternatively in the case of rats from a deficiency of vitamins A, D, or one or more of the thermostable B factors; in the case of birds it may result from a B_3 deficiency. In the case of less-studied animals, such as dogs, it is quite likely that loss of appetite may occur from a deficiency of these or still other less known factors, *e.g.*, the black tongue factor. In general, however, the indications are that a deficiency of thiamin leads to a rather more prompt loss of appetite than is the case with the other deficiencies. Certainly the administration of thiamin to an avitaminotic animal results in a very prompt and great stimulation of the urge to eat. It is reasonable then to speak of the vitamin requirement as that amount which is necessary to maintain appetite. However, we wish to emphasize that the requirement for appetite, maintenance, or growth of the young or weight maintenance of the adult may well be and probably is significantly different from the thiamin requirement defined as the minimum quantity necessary to prevent polyneuritis.

We are inclined to take exception to the use of the word *specific* in connection with the effect of thiamin on appetite. Certainly thiamin is not specific for appetite in the sense that the sex hormones are specific for sex functions or insulin is specific for diabetes. It appears that a thiamin deficiency impairs many functions and that it does so because it impairs a metabolic process which is essential to the proper nutrition of cellular tissue in general.

The importance of thiamin to appetite has been stressed by many writers and by none more than Cowgill (16). In his studies of the B_1 requirement of animals Cowgill often used anorexia as a criterion of thiamin deficiency and strongly emphasized the appetite-provoking qualities of this vitamin. They are undoubtedly extremely conspicuous. One cannot cease to marvel at the abrupt and large rise of voluntary intake of food by a polyneuritic animal upon administration of a substantial dose of thiamin. Often more food will be consumed and utilized within

twenty-four hours than the animal has taken during the entire previous week on the deficient diet. These responses are so marked that they can indeed be used readily as a rough measure of the thiamin administered. And yet it is scarcely proper to refer to this as a "specific" effect of thiamin as Cowgill and Sure do (17). The administration of a like amount of thiamin to a normal animal has no marked influence on its appetite. Only when a greater or less deficiency pre-exists does the effect upon appetite appear. Many workers with vitamin concentrates testify that self-administration of thiamin stimulates their appetites. It has even been recommended to the present writers as an appetizer before meals. Any such effect is considered to be purely fanciful unless, of course, the individual's existing supply is suboptimal. We have never been able to observe it. Further, as we have noted above, loss of appetite may be due to any one of a number of deficiencies. The promptness of development of anorexia in case of thiamin deficiency is merely a reflection of its rapid dissipation from the tissues by destruction within the body and by urinary excretion (see Chapter XIX).

Anorexia in thiamin deficiency is due, we believe, to failure of nutrition of the cells of the tissues. There exists in the body of polyneuritic animals considerable amounts of unmetabolized food. This may be viewed as causing loss of appetite for essentially the same reason that a full meal causes loss of appetite, namely, that the body is already surfeited with food. In case of thiamin deficiency the body lacks the means to metabolize it. This view is entirely consistent with the discussion in the preceding chapter on thiamin requirement in relation to quantity of food consumed. The appetite is, however, such a useful criterion of the state of well-being that thiamin requirement with respect to it has a profound importance in public health and especially in the nutrition of children.

A very interesting observation has recently been reported on the natural craving of B_1 deficient rats for even the synthetic thiamin (18). According to these authors solutions of the vitamin were offered to deficient rats side by side with as many as twelve other bottles containing solutions of other substances, some of them supposedly attractive in character. The rats promptly found the thiamin bottles and consumed as much as 11 cc. containing 1500 γ of thiamin within a half hour and three times that amount in twenty-four hours. The odor and taste aroused great interest, and the rats clung to the bottle with their paws and resisted its removal. This behavior appears somewhat at variance with another study of the appetite of rats as a guide to their choice of food to supply a deficiency (19). In this instance the rats seem to seek out a foodstuff which had proved beneficial from their past experience, identifying it by smell, taste, appear-

ance, etc. The choice was good when thiamin-rich foods were offered, but they were unable to discriminate when the thiamin content was relatively low so that little or no immediate benefit was produced. The rat was thus unable to distinguish a barely adequate diet from an inadequate one.

Thiamin in Special Relation to Growth

Rat growth as a method of testing the thiamin content of foods has been discussed at some length in Chapter XVII. The question has often been raised as to whether the marked effect of thiamin on growth is due merely to stimulation of appetite or also to an increased efficiency in utilization of food. That the latter is the case has been the conclusion of several workers and appears to us to be well justified. Some experiments of this sort have been reported recently, together with reference to earlier work (20). In this study groups of three young litter mates of the same sex were fed on the same diet except that one received autoclaved yeast, another raw yeast, the food intakes of the two being kept equal; the third received the same raw yeast diet as the second but its food intake was unlimited. The first made no growth in fourteen weeks, the second gained 50 grams, the third about 100 grams. This triad was typical of many which were subjected to the test. From these results it is concluded that thiamin produces growth in two ways, first by stimulating the appetite so as to increase food consumption and second by a "specific influence on growth, unrelated to the plane of nutrition." Similar results have been reported by Hogan and Pilcher (21), and we have had an ample experience confirmatory of them. However, the effect is scarcely a *specific* effect on growth. Administration of thiamin to full-grown animals whose supply is suboptimal likewise results in better utilization of foods as well as increased appetite and increased food consumption. Thiamin is specifically needed for the metabolic processes; in the young growth progresses or lags with the metabolism of the food.

If thiamin were a specific for growth one would expect the requirement of the young to be high as compared with that of the adult. This is not known to be the case. Young rats can grow for considerable periods on low intakes of thiamin, but as their size and need for food increases, growth halts unless larger amounts of thiamin are supplied. In the human infant beriberi is of frequent occurrence during the nursing period when the prevailing dietary of the mother is deficient in the essential vitamin, but once past the nursing age, children are conspicuously free from the disease. In the case of the nursling there is good reason to suppose that the mother's milk is toxic (see page 363) as well as deficient. On the whole, childhood appears to need

a lesser proportion of thiamin in the food than maturity. This appearance may well be false (see page 263). Probably the need for normal performance is nearly the same in both; it is certainly not materially larger during the growing period.

Two studies of recent date on thiamin intake in relation to development of children should be mentioned at this point. The later study (22) involved 193 normal infants living in their own homes. They were divided into two groups. One group received evaporated milk in which was incorporated an extract of rice polishings, the other an unfortified milk. The proportion of thiamin in the former was 3 to 5.4 γ per gram of solids, in the latter 1.0 to 1.5 γ. The diets of the two groups were otherwise the same. Water, corn sirup, and lemon juice were added to the milk. At four to five months milled cereal was also given; at five to six months vegetables; at six to seven months, fruit pulps, at eleven months, eggs. Cod liver oil was also supplied.

The infants on the diet higher in thiamin made somewhat more consistent and regular gains in weight than the controls, but the average weight and height at corresponding ages were not significantly different. Constipation and anorexia were equally absent in both groups. Respiratory infections were equally distributed and progress in sitting, walking, and talking was alike. Numerous anthropometric measurements and hematologic studies also failed to reveal significant discrepancies. The gain in weight per ounce of milk consumed was slightly higher for the higher thiamin group after the 126th day of life. This, we note, closely corresponds to the introduction of milled cereal into the diet, which may be significant. The sole definite physical benefit of the higher thiamin intake was a measure of stabilization of the growth rate. The thiamin intake of the group on the unfortified milk appears to be well above the amount needed to protect adults from beriberi (see Chapter VII). We may assume that the introduction of milled cereal reduced the thiamin-calorie ratio nearly to the minimum safe level, but the later use of vegetables, fruits, and eggs presumably raised the ratio again to some extent.

The observations on mental development of these infants are of interest. At the six-month level the high-thiamin group showed a definite acceleration in such tests as visual pursuit, eye-hand co-ordination, and prehension. The general picture was summarized as one of slightly accelerated maturation. At nine months the difference was somewhat less marked, and at twelve months still less so.

An earlier study (23) of children of five to nine years brought out the observation that every child showed striking improvement in each of several standardized mental tests applied before and after augmenting the "vitamin B" in the diet. Hoefer

and Hardy (24) correlated the intelligence quotients of public school children with their infant histories. Those breast fed as babies from the first to twentieth months were lowest in rank, those entirely bottle fed were next, and those breast fed up to four to nine months were first. Some connection between these observations and the low thiamin content of breast milk was surmised to exist. In this connection it will be well to record the earlier observation of Maurer and Tsai (25) that thiamin-deficient rats showed somewhat impaired maze-learning ability as compared with controls. Bernhardt (26) confirmed the finding, but others were not able to detect such an effect (27).

Another study of the effect of adding to the thiamin content of the diet of children in the form of wheat germ or brewers' yeast failed to reveal any physical benefit during the first year of life (28). A group of 238 infants living in homes in the neighborhood of Bellevue Hospital, New York, were used as subjects. Obviously such studies are very difficult to control, and some conflict of findings is not surprising.

The thiamin content of human milk is of great practical importance, not only in connection with pediatrics but also from the theoretical point of view, as it furnishes another much-needed means of ascertaining the human requirement of thiamin. The basis for such a determination is the undoubtedly valid assumption that well-fed human mothers must on the average secrete a food adequate for their young. We may, therefore, well review what is known of the thiamin content of human milk. A series of studies by Macy *et al.* is important (29, 30). They brought out that the pooled milk from a group of women was required in amounts of from 25 to 35 cc. daily to supply sufficient vitamin B complex for the growth of rats at the rate of 15 grams per week. Assuming thiamin to be the limiting factor, this should approximately correspond in thiamin content to 0.15 to 0.20γ of thiamin per cc. as compared with about 0.45γ per cc. for cow's milk. Because of uncertainties of conversion it is safer to be less explicit and to say that human milk appears to be roughly half as rich in thiamin as cow's milk. Reproduction of the rats which received human milk as the sole source of B vitamins was normal, but lactation was uniformly unsuccessful. The milks of two individual mothers were also studied. Both were somewhat lower in apparent thiamin than the pooled milk, but their babies gained at a rate in excess of the usual one. The diets of both women included large amounts of milk and seemed adequate in quality and high in quality, viz., 3500 to 5000 calories per day.

Later some further attempt was made to distinguish thiamin from the thermostable B factors by including autoclaved yeast

in the diet of the test rats (31) used in assaying breast milks from three additional subjects. As the autoclaved yeast was added to the diet during the course of the test, it was possible to note a slight upward inflection of the curve due to it, thus indicating that the thermostable factors supplied by human milk were also somewhat insufficient for the rat in the doses given. The growth rates and other circumstances of the assays, however, do not justify amplification of the above estimate of the thiamin content of human milk. There was an appreciable variation of thiamin content of the milks of the three women, the content being inversely proportional roughly to the volume of milk secreted daily by each.

During a subsequent period the women were given 10 grams of yeast daily and the milk reassayed. A slight enhancement of growth rate of the rats was noted though the food consumption was in general substantially lower than in the case of the rats used previously for the assay of the corresponding milks. The authors state that the substance supplied to the human milk by the yeast added to the diets of the women "is not an appetite stimulator; it is consequently improbable that it is the antineuritic vitamin." We regard this conclusion as very doubtful. Indeed, since thiamin is known to increase the efficiency of utilization of food under similar circumstances, one could well believe the contrary. However, the matter is quite uncertain. The opinion which is now widely held, based upon this work, that human milk cannot be enriched in thiamin content by enrichment of the mother's diet, is certainly not very well founded. Further experiments should be carried out with more liberal and known addenda of thiamin to the mother's diet. The yeast used in these experiments may have supplied relatively small amounts. It appears to have been a baker's yeast. Also, the method of assay of the human milk could be much improved from a quantitative standpoint by incorporating an adequate amount of autoclaved yeast in the diets of the rats from the outset. The women's babies in the above experiments showed no inflection in growth rate, nor did the growth rate of any one deviate significantly from those of the others.

Thiamin in Relation to Lactation

Since the metabolic rate rises in pregnancy (32) and especially during lactation, it might be expected that the thiamin requirement would rise in these conditions. This very definitely is indicated by clinical experience. In Manila signs of deficiency are noted in the great majority of women of the poorer classes during the childbearing age.

Evans and Burr (33) made a special study of the thiamin re-

quirement in relation to lactation in rats, using a diet of the usual synthetic type composed of casein, cooked cornstarch, and lard, with addenda of a salt mixture, cod liver oil, and wheat oil. Rats were found to grow and reproduce normally when supplied with 0.6 gram of yeast daily. However, during lactation the young failed to grow normally, especially during the period immediately before normal weaning, unless five times as much yeast was supplied. They concluded that B_1 was exclusively involved as the increased rate of growth of nurslings could be secured by adding rice polish extract, which contains little of some of the thermostable factors, to the mother's diet instead of additional yeast. A growth of 570 per cent in weight from birth to the twenty-first day of age was taken as the norm of good performance.

Sure (34) also studied the matter. His method was to transfer mothers from the stock-breeding diet to a synthetic B_1 deficient type upon the birth of young. Various mothers were supplied with addenda of rice polishings or autoclaved yeast or the two in combination. By noting changes in trend of the weight curves of the nurslings, following such additions to the diet, Sure concluded that more autoclaved yeast also benefited lactation. His experiments were less systematic in character than those of Evans and Burr, the feeding periods on each level of addendum being relatively short so that effects might well carry over from one period to another. It is also to be noted that his lower levels of supply of autoclaved yeast were below those which later experience has indicated to be necessary for proper growth of young rats. To obtain the maximum growth of nurslings, Sure found it necessary to use 20 to 30 per cent of yeast in the diet. This compares with approximately 15 per cent autoclaved yeast now generally recognized as necessary to supply adequate amounts of the thermostable factor. In this instance, as in many other experiments, a repetition of the work with addenda of pure thiamin in place of the poorly standardized products of natural origin which have heretofore been available will aid greatly in clearing up remaining uncertainties.

Further pursuit of the subject by Sure and associates has served to associate the beneficial action of B factors on lactation somewhat more definitely with thiamin. We can only refer to a few of the more recent papers. Sure and Walker (35) compared the effects of autoclaved yeast and unautoclaved yeast as addenda to the diet of mother rats during the period of lactation. The pairs which were compared as to the effect of the two addenda were litter mates and were transferred to the experimental diets on the day of the birth of their young. The rats on the raw yeast were restricted to the same amount of

food as was consumed by those on the B_1 deficient diet. The litters of the former showed a much higher growth and a much lower mortality than the litters of their mates on the deficient diet. The mortality of the latter was 70 to 100 per cent compared with complete success in the rearing of the young of the rats receiving raw yeast. Such an experiment is not convincing as to effect on lactation. It may merely mean that because the young lacked thiamin they died of a condition corresponding to human infantile beriberi. We can, however, accept the conclusion with some confidence that lactation increases the thiamin requirement on the ground that food consumption is greatly increased. Whether or not their need for thiamin is enhanced more than in proportion to the food consumption must await more accurate quantitative studies.

In another paper Sure (36) estimates that the lactating mother wastes at least 60 per cent of the daily thiamin intake as far as the quality of the milk is concerned. The large amounts of thiamin required for lactation are accordingly supposed to be due to the inability of the lactating mother to secrete the vitamin quantitatively in the milk. This point has also been stressed by I. G. Macy *et al.* in their studies of human milk, as well as by Evans and Burr, *loc. cit.*

Thiamin and Intercurrent Disease

Many experimental attempts have been made to connect thiamin deficiency with resistance to specific infectious disorders. The results have not been very impressive. Nevertheless, in classical studies of human beriberi striking evidence has been brought forth that serious disturbances of health of varied type are promoted by a restriction of diet to food sources which supply insufficient thiamin. It cannot be said definitely that this aggravation is due directly to thiamin shortage, as other deficiencies may have been involved. The results are, however, suggestive of such a relationship. Fraser and Stanton, in the course of conducting their deservedly famous experiment on the effect of polished and unpolished rice in coolie labor camps, noted that while beriberi did not appear until three to four months after the introduction of polished rice, the incidence of sundry other diseases leading to disability for work rose much more promptly.

It should be further pointed out that the thiamin requirement during periods of severe exertion and possibly during the course of intermittent fever such as malaria may rise considerably above the level adapted to other conditions.

Likewise, Baron Takaki also noted a correlation of beriberi and other disability in the Japanese army: ". . . the percentage of

kakke (beriberi) cases was a good indication of the general health of the army, because whenever kakke cases diminished the health of the army also improved generally, that is to say, cases of other disease decreased proportionally." In the aggregate there is strong reason for believing that the current world-wide incidence of beriberi by no means represents the sum total of thiamin insufficiency of the race. Some degree of inadequacy of supply of this vitamin probably is affecting adversely the health of a many times larger number of individuals.

The reader will have noted that a preponderance of the experiments referred to in this chapter have been performed with crude products containing thiamin. Repetition of many of them with the use of pure thiamin would be fruitful. The physician stands in great need of further evidence as to the effects of special circumstances on the thiamin requirement. Probably most of the subclinical forms of vitamin B_1 deficiency are to some extent due to the existence of special and unusual needs (see Chapter IV).

Summary

With certain diets growth may be enhanced somewhat above normal by adding large amounts of thiamin to the diet. With certain other diets this effect is quite absent. The effects of thiamin, therefore, are sometimes greatly dependent upon unknown factors in nutrition. Choline may be one of the important factors. Thiamin has a profound effect upon the appetite of animals which have subsisted on diets containing suboptimal amounts of it. It also promotes the efficient utilization of food and therefore promotes growth in the young and increment of weight in the adult.

A more generous supply of thiamin is necessary to proper lactation than is required for ordinary nutrition. The enhanced requirement is probably roughly in proportion to the enhanced food consumption of the mother.

None of the above effects is regarded as a specific property of the vitamin but as a secondary consequence of its role in food metabolism.

High intakes of thiamin (100 γ daily) have been reported to interfere with the proper rearing of young rats when the mothers are fed on a certain mixed diet of natural foodstuffs, but on a mixture of whole wheat and milk the rearing of young was normal. Further experiments are needed.

Human milk has been reported to be lower in thiamin than cow's milk and little dependent upon the thiamin content of the diet. Further work is needed to reach satisfactory conclusions as to the relative importance of deficiencies of thiamin and of the thermostable B factors.

Thiamin requirement in human infancy is probably not materially different from that in maturity except as the food consumption increases with increasing size.

Some association of intercurrent infections with thiamin deficiency is indicated by clinical experience. Resistance to specific infections is not demonstrably lowered in thiamin deficiency.

BIBLIOGRAPHY

1. Waterman, R. E., and Ammerman, M.: Studies of Crystalline Vitamin B. V. The Effect of Graduated Doses on Growing Rats. J. Nutrition *10*, 35, 1935.
2. Williams, R. R.: A Note on the Effects on Pigeons of an Exclusive Diet of Rice Meal, Bran and Polish. Biochem. J. *21*, 1349, 1927.
3. McCollum, E. V., Simmonds, N., and Pitz, W.: Nature of the Dietary Deficiencies of the Wheat Embryo. Jour. Biol. Chem. *25*, 105, 1916.
4. McHenry, E. W.: Effect of Choline on the Weights of Young Rats. Jour. Physiol. *85*, 343, 1935.
5. McHenry, E. W.: Vitamin B_1 and Fatty Livers. Jour. Physiol. *89*, 287, 1937.
6. McHenry, E. W.: An Effect of Choline on the Vitamin B_1-sparing Action of Fats. Biochem. J. *31*, 1616, 1937.
7. Elvehjem, C. A.: Private communication.
8. Elvehjem, C. A., and Arnold, A.: Interrelationships of Vitamin. Nature *137*, 109, 1936.
9. Leong, P. C.: Vitamin B_1 in the Animal Organism. II. A Quantitative Study of the Metabolism of B_1 in Rats. Biochem. J. *31*, 373, 1937.
10. Sampson, W. L.: Private communication.
11. Rigler, R.: Über wirksame Stoffe in Herzhormonpräparaten. Med. Klin. *24*, 574, 1928.
12. Zwaardemaker, H.: Formation of Automatins from Vitamin B by Radioactive Radiation. Compt. Rend. Soc. Biol. *98*, 183, 1928.
13. Zwaardemaker, H.: Radiated Vitamin B and Automatin Action. Proc. Roy. Acad. Amsterdam *31*, 258, 1928.
14. Wenckebach, K. F.: Heart and Circulation in a Tropical Avitaminosis. Lancet II, 265, 1928.
15. Perla, D.: Toxic Effects of an Excess of Vitamin B_1 in Rats. Proc. Soc. Exp. Biol. Med. *37*, 169, 1937.
16. Cowgill, G. R. Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
17. Sure, B.: A Detailed Study of the Rôle of Vitamin B_1 in Anorexia in the Albino Rat. J. Nutrition *1*, 49, 1928.

18. Richter, C. P., Holt, L. E., and Barelare, B.: Vitamin B_1 Craving in Rats. Science *86*, 354, 1937.
19. Harris, L. J., Clay, J., Hargreaves, F. J., and Ward, A.: Appetite and Choice of Diet; the Ability of the Vitamin B Deficient Rat to Discriminate between Diets Containing and Lacking the Vitamins. Proc. Roy. Soc. Lond. *B 113*, 161, 1933.
20. Sure, B., Kik, M. C., Walker, D. J., and Smith, M. E.: Dietary Requirements for Fertility and Lactation. IV. The Specific Effect of Vitamin B on Lactation and Growth. Univ. Ark. Agri. Exp. Sta. Bull. *284*, 1933.
21. Hogan, A. G., and Pilcher, R. W.: Effects of Variations in the Amounts of Vitamin B and Protein in the Ration. Univ. Missouri Agri. Exp. Sta. Research Bull. *195*, 1933.
22. Colby, M. G., Macy, I. G., Poole, M. W., Hamil, B. M., and Cooley, T. B.: Stabilizing Effect of Increased Vitamin B (B_1) Intake on Growth and Nutrition of Infants. Relation of Increased Vitamin B (B_1) Intake to Mental and Physical Growth of Infants. Amer. Jour. Dis. Child. *54*, 726, 750, 1937.
23. Balkin, E. R., and Maurer, S.: Variations in Psychological Measurements Associated with Increased Vitamin B Complex Feeding in Young Children. Jour. Exper. Psych. *17*, 85, 1934.
24. Hoefer, C., and Hardy, M. C.: Later Development of Breast Fed and Artificially Fed Infants. Jour. Amer. Med. Assn. *92*, 615, 1929.
25. Maurer, S., and Tsai, L. S.: The Effect of Partial Depletion of Vitamin B Complex upon Learning Ability in Rats. J. Nutrition *4*, 507, 1931.
26. Bernhardt, K. S.: Effect of Vitamin B Deficiency during Nursing on Subsequent Learning in the Rat. J. Compt. Psych. *17*, 123, 1934.
27. Anderson, J. E., and Smith, A. H.: The Effect of Quantitative and Qualitative Stunting on Maze Learning in White Rats. J. Compt. Psych. *6*, 337, 1926.
28. Elias, H. L., and Turner, R.: A Clinical Study of the Influence of Vitamin B Supplements. Jour. Pediat. *8*, 352, 1936.
29. Macy, I. G., Outhouse, J., Long, M. L., and Graham, A.: Human Milk Studies. III. The Quantitative Estimation of Vitamin B. Jour. Biol. Chem. *73*, 189, 1927.
30. Macy, I. G., and Outhouse, J.: Breast Milk—a Variable Food. Jour. Amer. Dietetic Assn. *4*, 9, 1928.
31. McCosh, S. S., Macy, I. G., and Hunscher, H. A.: Human Milk Studies. VI. Vitamin Potency as Influenced by Supplementing the Maternal Diet with Yeast. Jour. Biol. Chem. *90*, 1, 1931.
32. Rowe, A. W., and Boyd, W. C.: The Metabolism in Pregnancy. IX. The Foetal Influence on the Basal Rate. Jour. Nutrition *5*, 551, 1932.
33. Evans, H. M., and Burr, G. O.: On the Amount of Vitamin B Required during Lactation. Jour. Biol. Chem. *76*, 263, 1928.

34. Sure, B.: A Differentiation of the Vitamin B Complex in Rice Polishings as Evidenced in Studies of Lactation. Jour. Biol. Chem. *80,* 297, 1928.
35. Sure, B., and Walker, D. J.: The Specific Effect of Vitamin B on Lactation. Jour. Biol. Chem. *91,* 69, 1930.
36. Sure, B.: The Inefficiency of the Lactating Mother (*Mus norvegicus albinus*) in Secreting Vitamin B in the Milk and the Relation of this Phenomenon to Infant Mortality. Jour. Biol. Chem. *76,* 685, 1928.

CHAPTER XXIII

THIAMIN REQUIREMENT IN RELATION TO WEIGHT

THE principal study of this subject in the literature is that of Cowgill (1). His study begins with pigeons and involves the application of the principle of maintenance of appetite, which is in turn gauged by the maintenance of weight. This standard of B_1 requirement has already been discussed in Chapter XXII. Cowgill's method was to feed pigeons *ad libitum* upon polished rice, stone grit, and water. After two weeks this diet was supplemented by meat residue, salt mixture, and two to four drops of cod liver oil daily. At the time of transfer of the pigeons from the first to the second diet, they were classified according to their body weights into three groups and dosed daily with a B_1 supplement graduated according to the average group weights. After a period of adjustment with various doses of the B_1 supplement, a dosage was arrived at at which each bird was found to maintain fairly constant weight over a period of one to three weeks. The size of the dose required in each case to maintain this constancy of weight versus the average body weight of the individual bird during the period of constancy was noted. The conclusion was reached that the vitamin B requirement is proportional to $W^{\frac{2}{3}}$, where W is the average weight of the individual for the period of test.

These experiments have several serious defects which are now discernible in the light of knowledge gained in subsequent years about the multiplicity of B factors and their effects on nutrition. First: The pigeons' need of B_3 for weight maintenance is not provided for except as the B_3 was present incidentally in the B_1 preparation used. This was lot 985 of Harris yeast vitamin powder, which we have every reason to suppose contains more or less B_3. We do not know, however, whether B_1 or B_3 was the limiting factor in the powder. Second: Cowgill did not determine the amount of B_1 required to maintain the birds at normal weight but at some undefined subnormal weight. Evidently this weight was considerably below the normal from the two curves which have been published. Apparently the normal weight was not ascertained nor was there any effort to assure that each bird was

equally subnormal in weight at the outset of the dosage period (2). Waterman and Ammerman (3) have shown that pigeons on autoclaved wheat plus 5 γ of pure B_1 daily maintain a weight level of about 72 per cent of normal and that this weight level rises only to about 85 per cent of normal even when 40 γ of B_1 daily are given (see Fig. 14). It is thus evident that if the B_3 supply in the Harris yeast powder as given were insufficient, the apparent B_1 requirement would rise very rapidly with the weight level at which it was decided to maintain any given bird. Also, if the birds were unequally lean when the dosage began, the dose

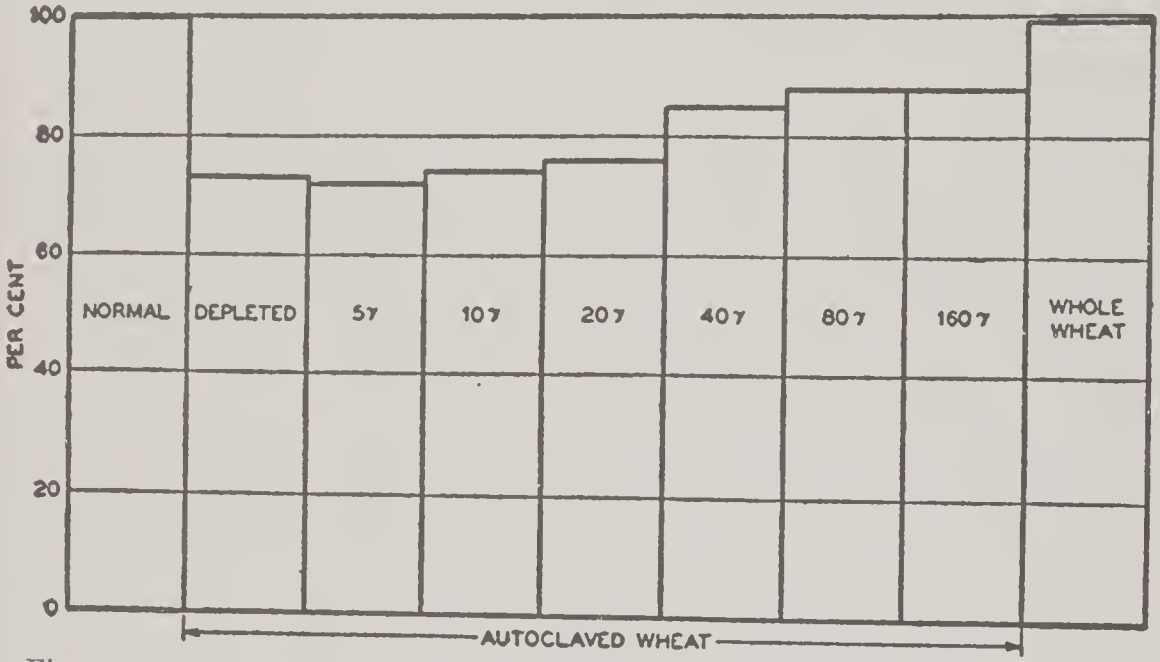


Figure 14. Average weight level of pigeons on a diet of autoclaved wheat plus various doses of crystalline vitamin B_1 hydrochloride. Courtesy of *Journal of Nutrition*.

required to maintain the existing weight level would depend primarily upon the initial leanness and not on the skeleton size or normal or potential weight of the bird. The pigeon experiments should, therefore, be heavily discounted.

Cowgill's study was extended to three other species. In the case of rats the method was to feed young animals of 60 grams' body weight on a typical diet of commercial casein, cornstarch, lard, and butter fat, supplemented by salt mixture and lot 985 yeast vitamin powder, in definite doses, just sufficient to produce a more or less normal growth. This dosage was continued until the growth rate showed a decline, whereupon the dosage was increased and so maintained until the growth rate again began to fall; a second increase in dosage was then put into effect. The amount of yeast powder associated with each weight level was noted and was found to be approximately proportional to $W^{\frac{5}{3}}$.

In these experiments no autoclaved yeast or other ample source of the thermostable B factors was supplied, as is now

customary. The Harris yeast powder, of course, contains these factors, but not in sufficient amount at the levels of feeding, viz., 20, 30, and 45 milligrams per day. This is clearly shown by the fact that the growth rate could not be maintained at levels above 80 to 108 grams when the yeast powder dosage was 20 milligrams daily. This amount of yeast powder is equivalent to 4–5 γ of thiamin chloride, which, as is well known, is able to support good growth far beyond this weight level if the diet is otherwise adequate. It is also to be noted that the larger doses of yeast powder were given at later times, that is, after the animals had been further depleted of the thermostable factors. It is now well known that the rate of depletion of the body of the thermostable factors is much slower than that of B_1. It was, therefore, inescapable that more yeast powder would be required to produce growth at the weight levels later attained, as the need for thermostable factors had then become more acute. The greater weight at later stages was also due in part, no doubt, to greater age, certainly not wholly to the greater B_1 supply. The experiments would have accordingly been better in this respect had they been run simultaneously with parallel groups and not consecutively on the same animals.

Harris yeast powder supplies both B_1 and the thermostable factors, but certain of the latter are present in only one-fourth as great a proportion relative to the animals' need. The ratio is 240 Sherman-Chase units of B_1 to 60 Sherman-Bourquin units of thermostable (G) (4). Since both units are defined as the quantity of each of the two respective factors necessary to maintain a rate of growth of 3 grams per week, the units afford a direct comparison of adequacy with respect to the two factors. It is thus clear that the thermostable B factors, not B_1, were the principal limiting elements in Cowgill's rat experiments.

The mice experiments (5) are similar in character. Young mice were fed upon a diet essentially the same as that used for the rats with the exception that the casein was purified by extraction and thus more fully freed of B factors. Two types of experiment were involved. In one the mice were given a daily supplement of 5 milligrams of 985 yeast powder, which was later increased in eight successive steps to about 70 milligrams daily. The growth attained throughout the entire period was substantially subnormal, a reflection primarily of the fact that the thermostable deficiency was accumulating through the growth period. In the other type of experiment with mice, groups of young animals were depleted for a short time and then given a fixed dose daily of the yeast powder during the remainder of the experiment. The dose varied from group to group from 10 to 50 milligrams. In the latter experiments the weight ultimately attained on a given dosage was tabulated against the dosage as

an index of the relationship of B_1 requirement to the weight. The deficiency of the thermostable factors was even more marked in the case of the mouse experiments as compared with those with the rat because of the purification of the casein. It seems probable that this is responsible for the fact that the best-fitting exponent of the weight in this instance was found to be nearer to 7/3 rather than 5/3, although Cowgill attributed it to having given the yeast powder in excess of the absolute minimum.

Regarding the dog experiments, we are not in a good position to comment at length because of lack of experience with this animal. Since in the dog experiments meat residue constituted a substantial fraction of at least one of the diets used, there was probably a sufficiency of at least some of the thermostable factors present. The requirement of the dog for the various vitamins has been much less studied than that of the rat, so that we know less of what factors may be needed. Symptoms are described in these experiments which appear to be of a character to be expected from B_1 deficiency. On the other hand, it is to be noted that although many other dogs were studied by Cowgill and his associates, a series of only six dogs was used to establish the relationship between vitamin requirement and weight. Detailed evidence is lacking as to the normal weights of the dogs. Cowgill and associates had previously reported that "dogs require approximately 40 milligrams of the yeast vitamin powder (Harris) per kilogram of body weight per day to maintain perfect appetites over periods of several months' duration." Later, in going over the same experiments in the light of the hint which they got from the pigeon experiments, the conclusion was reached that, as in the other species, the vitamin requirement for dogs is proportional to $W^{\frac{5}{3}}$.

We cannot accept this general conclusion. If the vitamin requirement is proportional to $W^{\frac{5}{3}}$, a 72-kilo Great Dane will require 63 times as much vitamin daily as a dog of 6 kilos, whereas its food consumption would be only 5.25 times as great. The Great Dane would require 392 times as much vitamin in 10.9 times as much food as a 2-kilo Pekingese would need. Its food supply would have to be 36 times as rich in vitamin as the smaller dog's.

The dog should lend itself particularly well to a further study of the question at issue because of the very wide range of weights which are represented. It is to be hoped that someone will conduct such an experiment, as we need to know whether a species constant exists. The test will probably have to be prefaced by a certain amount of experimentation to be sure that the diet used is adequate in every way aside from its B_1 content. B_1 should, of course, be supplied in crystalline form, since that is now readily available. In such experiments we wish to suggest the advis-

ability of destroying any residual B_1 contained in the various articles of food, not by a process of autoclaving, which is extremely likely to destroy other factors, but by a mild sulfite treatment which appears very likely to be a specific reagent for the destruction of B_1 alone.

Waterman and Ammerman have pointed out in a very clear fashion that in both the pigeon and the rat on certain types of diet the weight level of any given individual may be pushed up higher and higher by supplying more and more B_1. This was accomplished in the one case where B_3 deficiency was known to exist and in the other where no known deficiency existed, although some thermolabile deficiency is suspected. This forcing upward of the weight level of an individual, however, was accomplished in both species only by supplying manyfold larger quantities of the crystalline vitamin. The higher the level the larger the increment of vitamin necessary to achieve a new weight level or higher growth rate. We think it likely that in part Cowgill's results constitute a foreshadowing of the Waterman-Ammerman phenomenon. It is also foreshadowed elsewhere. Cowgill's results may come to assume greater importance with respect to B_1 requirement in relation to higher degrees of well-being than we can now foresee. However, the amounts of B_1 which are involved in such cases do not appear to be related to the minimum requirement for the prevention of outright beriberi when B_1 is the sole deficiency. Nor can we suppose that because an animal on a certain diet requires quantity a of vitamin to rise from weight level n to $n + 1$, another animal of the same species on the same diet whose weight is already $n + 1$ will also require quantity a to maintain that level. One is a question of leanness, the other a question of skeleton size.

The error in Cowgill's theoretical formula detracts very little from the value of his analysis of diets associated with beriberi or with recessions of the disease (see Chapter VII). The data are set forth so that one can conveniently and profitably read the latter two thirds of the book and exercise his own judgment about the acceptance or rejection of the feature regarding the effect of weight on the vitamin-calorie ratio required.

Elsewhere in the literature the indications are that the vitamin-calorie ratio is independent of the weight as far as the requirement for prevention of beriberi is concerned. If it depended upon the weight, one would expect that larger pigeons would be more subject to polyneuritis than smaller ones when all are fed on the same diet. In Figure 15 we have plotted the initial weights of ten birds fed simultaneously on a 71 per cent wheat flour (that is to say, 71 per cent of the whole grain was included in the flour) against the days elapsing before onset of polyneuritis (unpublished data). This flour was just deficient

enough in B_1 to produce a tardy polyneuritis. It was not far below the borderline in this respect, for in parallel experiments conducted simultaneously with an equal number of birds it was demonstrated that no polyneuritis occurred on a 79 per cent flour, while on a 49 per cent flour polyneuritis occurred much more quickly. Such a borderline diet as the 71 per cent flour should, therefore, be favorable for detecting any influence of

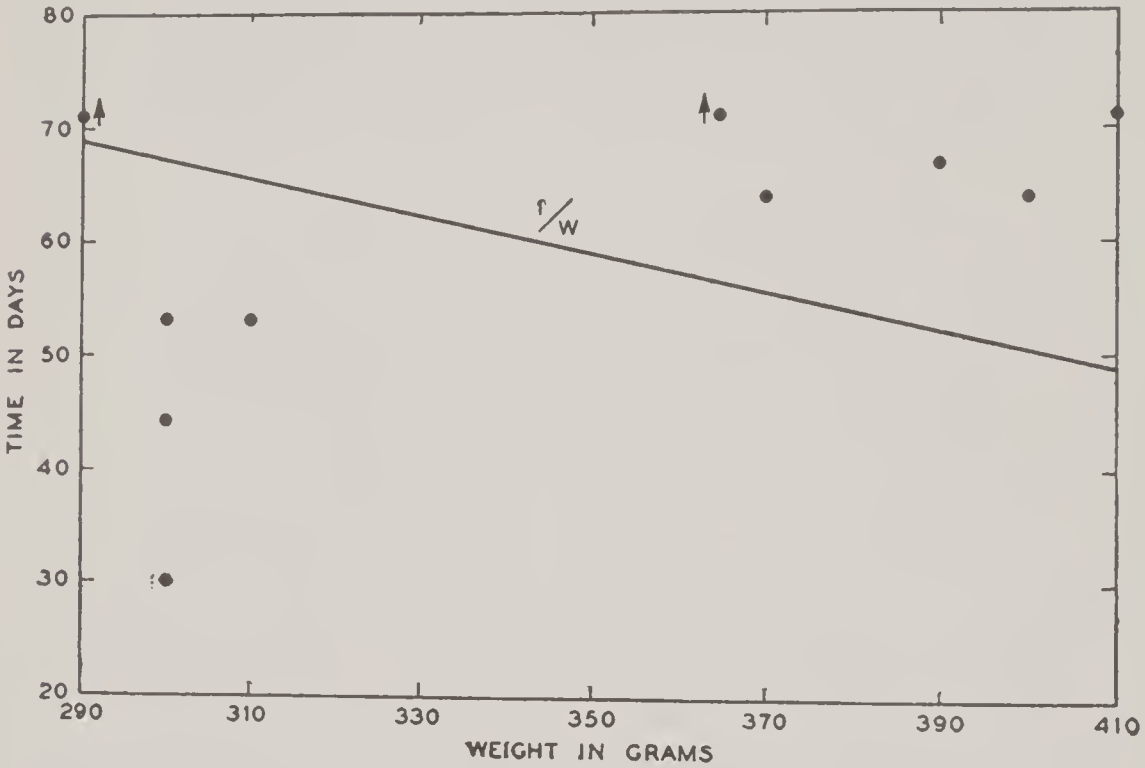


Figure 15. Relation of time of onset of polyneuritis to initial weights of pigeons. The diet was a 71% wheat flour. ↑ signifies that polyneuritis had not developed at the end of 72 days when the experiment was discontinued. The distribution should be parallel with the line 1/W to be in accordance with the supposed increase of proportional requirement with weight.

weight. As can be noted from the figure, there appears to be a slight tendency for the smaller birds to contract polyneuritis first, which is quite the reverse of Cowgill's prediction. We do not, however, regard this tendency as necessarily significant. It may be an accidental variation.

Numerous like experiments have been recorded by G. Grijns (6), who fed domestic fowls on various lots of polished rice with and without addenda of various sorts. A few typical results are plotted in Figure 16. There is no observable relationship between the weight and the rapidity of onset of the disease, nor have we been able to detect any such relationship in the large numbers of similar experiments with chickens which have been reported by Fraser and Stanton (7).

What is of particular interest in this connection is any relationship which may exist in the human species between body weight and B_1 requirement. The data which are available in

the literature are not abundant. We may refer to the experiments of Strong and Crowell (8) with prisoners in Bilibid prison at Manila. In these experiments seventeen men were confined to a uniform B_1 deficient diet. Of these the majority developed beriberi of greater or less severity, but there appears to be no relationship between the body weight of the individuals and the time of incidence or severity of the symptoms.

In many epidemics of beriberi which have been reported no evidence is available that all were restricted to a uniform diet. An exception is the cruise of the Japanese ship *Riujio* in 1883 with a complement of 376 men, of whom 169 contracted beri-

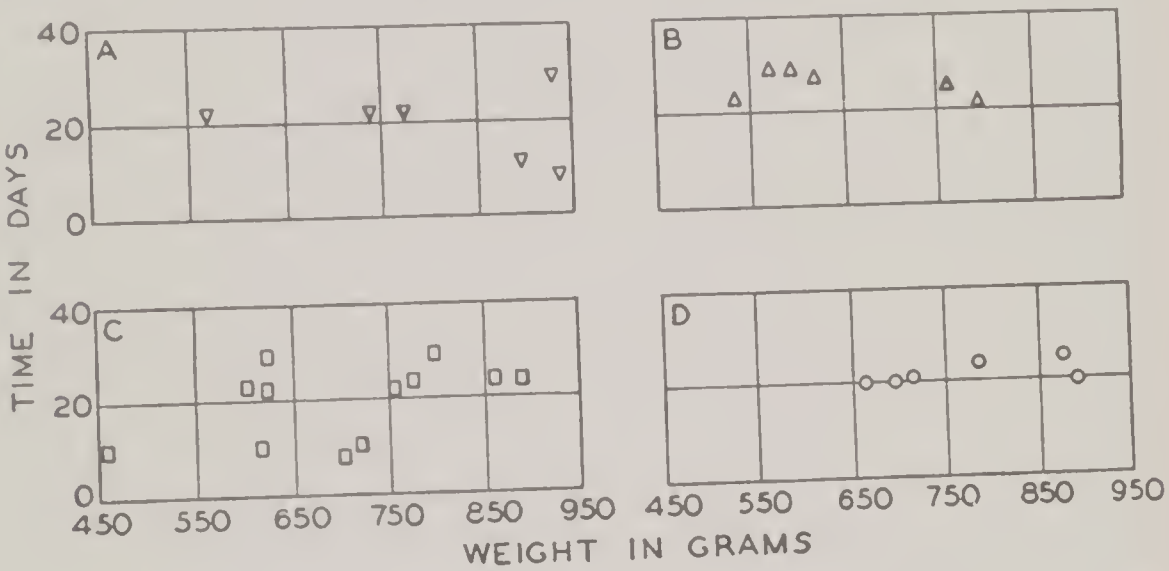


Figure 16. Each plot represents a group of fowls fed simultaneously on the same diet. In each case the diet consisted of a particular lot of boiled polished rice. In "B" a salt mixture was provided in addition.

beri during the course of 272 days. Another exception of more recent occurrence is the epidemic of beriberi among the British troops at the siege of Kut-el-Amara in 1915–16. We have endeavored, both by search of the literature and by correspondence, to secure data on the normal weights of the individuals exposed to these epidemics, but have not succeeded in doing so.

However, through the kindness of Dr. S. Ohdake of the Imperial University of Tokio, our attention was called to a very pertinent study by Kawaguchi (9). It pertains to an outbreak of beriberi in 1919–20 on the Japanese warship *Iwami,* stationed at Sagaren Island. Sixty-nine (69) cases of beriberi occurred among a complement of 720 men. Data are presented as to the seasonal variation in the average weight of the men. These are reproduced in Figure 17 as a matter of general interest. Kawaguchi noted that the epidemic of beriberi appeared at the period when the average weight of the men was approaching its annual maximum and remarked particularly on the contrast between human and animal beriberi in that the latter is preceded by loss rather than gain in weight. To support this finding he gives the

weight curves of each of the 69 men who contracted beriberi, showing a rise of weight prevailingly present prior to the onset of the attack, probably due to edema. In spite of the edema factor which would operate to increase preferentially the weights of the incipient beriberics, we are able to read from these curves the following figures which do not conform to Cowgill's contention that the vitamin-calorie ratio which is required to protect human beings against beriberi rises with the weight of the individual. Kawaguchi's data are particularly pertinent because the

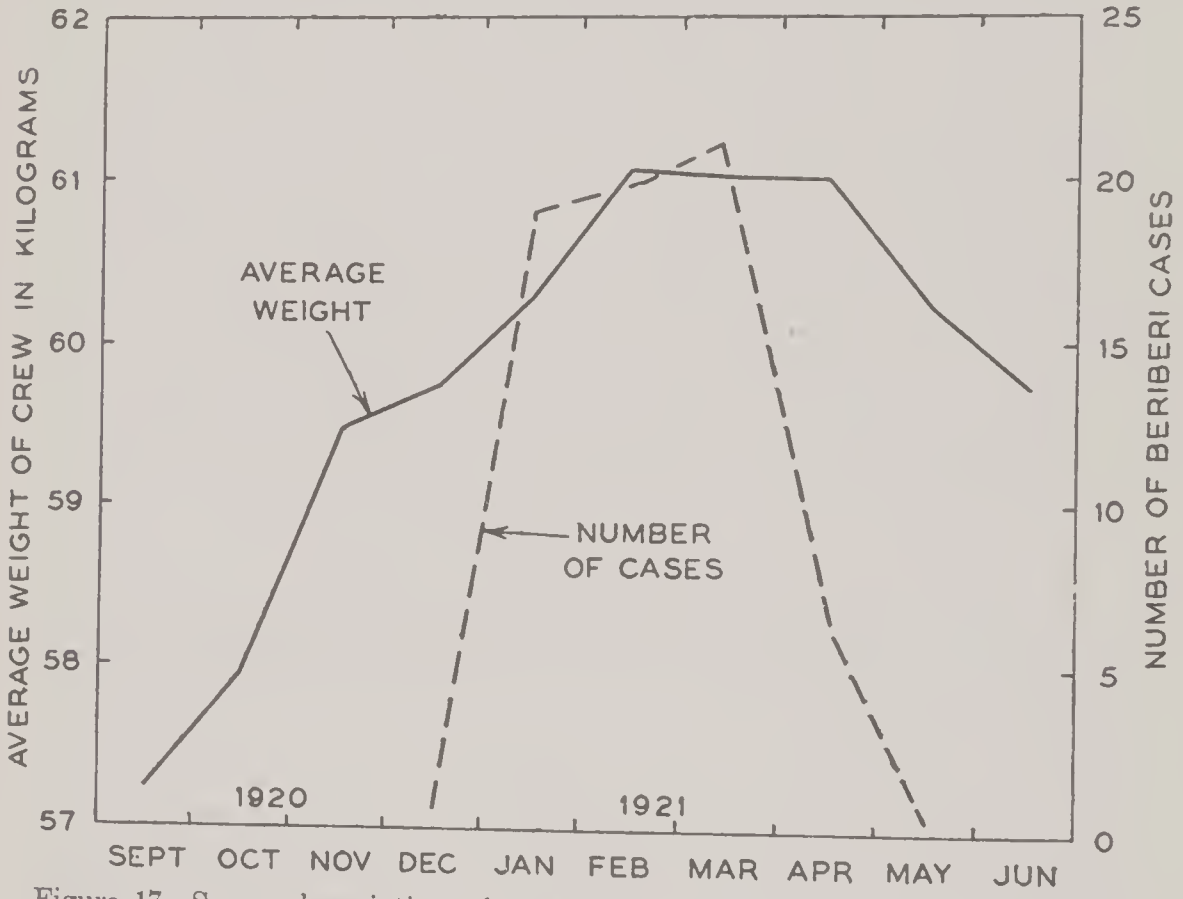


Figure 17. Seasonal variation of weight and incidence of beriberi in the crew of the *Iwami*, 1919–20.

total number of men involved (720) was sufficiently large to be treated statistically and because the incidence of beriberi (9 per cent) is low enough to demonstrate clearly that the diet was borderline with respect to B_1 deficiency.

The significant data are summarized below for the third month (September) preceding the beginning of the beriberi epidemic (December):

Weight of individuals who subsequently contracted beriberi	Maximum	72.4 kilos
	Minimum	49.9 kilos
	Mean	56.0 kilos
Number of subaverage individuals	36	
Number of superaverage individuals	32	
	68 *	
Average weight of 720 men		56.2 kilos

* The September weight is lacking for one of the 69 incipient beriberics. The weights for another month would serve as well, as the average weight curve of the incipient beriberics follows that of the whole crew quite closely.

If Cowgill's contention were correct that the required vitamin-calorie ratio rises in proportion to the weight, the required concentration of vitamin in the food would be 40 per cent higher for the large men (70 kilos) than for the small ones (50 kilos) and there should have been a marked preponderance of men of superaverage weight among those who later contracted beriberi. Actually the preponderance is slightly in the opposite direction. It is doubtful that this preponderance is significant, but it is perhaps well to call attention again to the tendency toward a higher metabolism in smaller individuals on account of the higher ratio of body surface to body weight. This is in harmony with a great weight of evidence in favor of the view that the B_1 required is in proportion to the quantity of food and particularly of carbohydrate metabolized.

It should be emphasized that the foregoing discussion of thiamin requirement in relation to weight has nothing to do with the question of whether persons who are disposed to be fleshy have a lower or higher requirement than those who are prone to be lean. This question should be considered in the light of the discussion in Chapters XX and XXII. Probably such factors as thyroid secretion play an important role, but little is known as to the position of thiamin on the stage.

Summary of Conclusions

The vitamin-calorie ratio required for a given species appears independent of the weight of the individual within the limits of expermental error.

BIBLIOGRAPHY

1. Cowgill, G. R.: Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
2. Klotz, B. H.: Doctor's Thesis, Yale University, 1926.
3. Waterman, R. E., and Ammerman, M.: Studies of Crystalline Vitamin B. VI. The Effect of Graduated Doses on Pigeons. Jour. Nutrition *10*, 161, 1935.
4. Harris, Dr. Isaac: Private communication.
5. Beard, H. H.: Studies in the Nutrition of the White Mouse. Amer. Jour. Physiol. *75*, 668, 1926.
6. Grijns, G.: Über Polyneuritis gallinarum. Genees. Tijds. Ned. Ind. *41*, 3, 1901.
7. Fraser, H., and Stanton, A. T.: The Etiology of Beriberi. Studies Inst. Med. Research Fed. Malay States, No. 12, 1911.
8. Strong, R. P., and Crowell, B. C.: Etiology of Beriberi. Phil. Jour. Sci. Sect. *B7*, 271, 1912.
9. Kawaguchi, Z.: On the Relation of Beriberi and Body Weight. Jour. Naval Med. Soc. Japan, p. 32–42, 1920–22.

CHAPTER XXIV

THIAMIN REQUIREMENT IN RELATION TO SPECIES

EVEN a superficial examination of the literature indicates a considerable variation in the B_1 requirement per unit of body weight from species to species. Cowgill (1) has made a more elaborate study of this matter than has been reported elsewhere. His analysis begins with the relationship between the weight of an individual animal and its B_1 requirement in four species, the mouse, the rat, the pigeon, and the dog. Using the criterion of maintenance of appetite, he finds that with any one species the B_1 requirement $= K_s W^{\frac{2}{3}}$, where K_s is a species constant and W is the weight of the individual. That is to say, according to Cowgill in a series of animals of the same species but of varying weights, the B_1 requirement necessary to maintain appetite rises much faster than the weight. This finding we believe to be entirely erroneous, for the reasons mentioned in the previous chapter.

However, Cowgill's conclusion in this regard does not necessarily affect directly the validity of his findings with regard to the relative magnitudes of the several species constants, that is, the proportionality factors relating vitamin requirement to weight for each species.

Assuming that he was able to apply the same criterion of appetite to each species on a strictly comparative basis, the relative magnitudes of the several species constants should be correct regardless of what power of the weight is involved or whether there is any universal constant for all the species. Cowgill plots the logarithm of the maximum recorded normal weight ever attained by an individual of the species against the logarithm of the several species constants. He obtains substantially a straight line, the equation of which can be written:

(1) $$Vit_i = 4.9 \cdot W_i^{.66} \cdot \frac{W_i}{W_{max}}$$

By further experiments on two species he arrives at the conclusion that the energy level above basal metabolism for the species in question is represented by:

(2) Calories per day $= 1.5\ W_i^{.66}$, whence

(3) $$W_i^{.66} = \frac{Cal_i}{1.5}$$

Substituting in (1) he obtains:

(4) $$\frac{Vit_i}{Cal_i} = \frac{3.27}{W_{max}} \cdot W_i$$

in which Vit_i is the daily vitamin requirement in milligram equivalents (1 mg. eq. $= 0.2\ \gamma\ B_1$) of an individual, W_{max} is the maximum weight in grams attained by the species, Cal_i is the daily food intake in calories, and W_i is the weight in grams of the individual. From (4) we obtain by substituting the indicated values of W_{max} assumed by Cowgill the following, in which Vit_i is expressed in micrograms (γ):

Species	W_{max}	$\frac{Vit_i}{Cal_i}$
Man	115,000	$0.0000057\ W_i$
Dog	72,000	$0.0000091\ W_i$
Pigeon	1,350	$0.00048\ W_i$
Rat	520	$0.00126\ W_i$
Mouse	34	$0.0192\ W_i$

One cannot avoid astonishment at this portrayal of a proportional requirement for the mouse which is more than 3000 times *larger* than that for man, while the proportional requirement for men or for mice is said to be *smaller* as size decreases.

The fact that Cowgill's species constants plotted against the maximum weight on a log-log basis give approximately a straight line is at first glance impressive. As one reflects about it, it becomes clear that the magnitude of any of the constants might be altered by 10 or 20 per cent in either direction without greatly impairing the fit of the points along the line, as a log-log plot is such as to conceal minor variations. The order of magnitude of the constants, however, could not be changed without ruining the fit. However, if the ratio, W_{mean}: W_{max}, is approximately constant for the four species, the straight line would be accounted for. If W_{mean} is taken as the mean weight of the animals used by Cowgill, these ratios become 20:34 = .59 mouse; 125:520 = .24 rat; 420:1350 = 0.31 pigeon; 8230:72,000 = 0.11 dog. The ratios are of the same magnitude and, therefore, do not constitute a critical test of the significance of the straight line in Cowgill's plot. In this plot the straight line fit is much better if one omits the dog or the mouse, preferably the latter. This is exactly the result indicated by the deviations from constancy among the above ratios. Had Cowgill worked, for example, with unusually small dogs and unusually large rats or mice or vice versa, the significance of a straight line would be much greater. As matters stand, the straight line appears to be fortuitous. One cannot feel

any assurance from these data that any universal constant exists, especially as the whole question is further complicated by multiple deficiencies, etc., involved in the experiments as discussed in the last chapter. It need only be noted here that the estimates of requirement under the conditions of Cowgill's experiments are definitely higher for both mouse and rat than when the diets are rendered more complete by supplying the thermostable factors in ample amount in the form of autoclaved yeast as has been done by other workers. The pigeon experiments are similarly complicated by lack of a uniform and adequate supply of B_3.

Quite apart from these calculations or speculations it appears that there is a tendency for the small species to require larger amounts of vitamin per unit of body weight than do the larger species. This is evident from the results of many workers and is also shown by direct experimental data taken from Cowgill.

Species	*Typical Weight gm.*	*Mg. Eq. Required Daily*	*γ Required Daily*	$\frac{W}{Vit}$
Mouse	20	20	4	5
Rat	125	30	6	21
Pigeon	420	86	17	25
Dog	8000	232	48	167

If a man of 60 kilos' weight required vitamin in the same proportion as the mouse, his daily need would be 6 to 12 milligrams, whereas the existing experimental evidence indicates that it is about 1 milligram.

However, in making this statement with regard to the B_1 requirement of man we have been compelled to use another standard than that which Cowgill utilized in his experiments with animals, namely, the requirement to prevent beriberi rather than the requirement to maintain appetite. It is, however, the same standard as Cowgill used in transferring his experimental results to the consideration of human dietaries in relation to human needs. This change of standard is possibly all-important. From what has been said in Chapter XXII with regard to the B_1 requirement with respect to state of well-being, one might readily infer that man might under some circumstances require several milligrams daily to maintain appetite and weight, but the examination of human diets makes it extremely unlikely that the human intake often approaches such a figure. In the light of this it may seem more reasonable to suppose that for man, at least upon the usual diets, the disparity between the amount of vitamin necessary to prevent beriberi and that required to maintain complete well-being is smaller than that which has been observed in rats, as, for example, in the experiments of Waterman and Ammerman using the Chase-Sherman diet (see

Chapter XXII). Alternatively one may ascribe the Waterman-Ammerman phenomenon to peculiarities of the Chase-Sherman diet or to contamination of the natural crystalline vitamin with traces of B_4. In either of these cases the result becomes anomalous and inapplicable to human diets and human behavior in general.

If we take Cowgill's value of 30 milligram equivalents (6γ) for a 125-gram rat as a basis, a 60-kilo man's proportional requirement would be 2.88 milligrams, which is surely rather high. Taking the Cowgill value for a 420-gram pigeon, namely 17 γ, man's proportionate requirement would be 2.43 milligrams, which is again high according to best available estimates. But taking a dog of 8 kilos, requiring 48 γ daily as a basis, man's requirement would be 1.25 milligrams, which is nearer right. All this is in accord with the general observation that the vitamin requirement per unit of body weight falls as one passes from smaller to larger species, as Cowgill has noted.

It can scarcely be doubted that this difference in requirement arises from the fact that the food consumption per unit of body weight is much larger for the smaller species. A man would consume 7 to 8 kilos of dry food per day if he ate in proportion to the rat. That the relationship of B_1 requirement to food consumption is precise or that the requirement varies in some uniform manner from species to species according to the characteristic weight is beyond the range of our present knowledge. The acceptance of the rough principle that the smaller species require more vitamin per unit of body weight does not depend upon the use of Cowgill's conditions of experiment nor the acceptance of his criterion of maintenance of appetite as defining the requirement. Entirely independent work shows that the rice bird, weighing about 30 grams, requires 2 γ of vitamin per day or one part per million of polished rice to protect it from polyneuritis. The pigeon, weighing approximately 300 grams and consuming normally about seven times as much food as the rice bird, requires only about four times as much vitamin daily for equal protection. Another reflection of the effect of species size in a series of related species is the fact as reported by Jansen and Donath (2) that rice birds contract polyneuritis in nine to twelve days on polished rice, while pigeons require twenty to thirty days and chickens more than a month. Presumably this statement was intended to apply to a single uniform lot of rice. Dr. E. B. Vedder has reported in a personal communication that sparrows develop polyneuritis on a polished rice diet in about twelve days. Similarly, Freudenberg and Cerecedo find mice developing polyneuritis in fifteen to twenty days (3), while rats require five to eight weeks. The variation from individual to individual, however, is considerable for both birds and rodents,

and one can scarcely express time required to develop polyneuritis at all precisely in terms of the weight. Later work of Cerecedo, Hennessy, Kaszuba, and Thornton (4) shows that mice require 2 γ of B_1 for "normal" growth, compared with 4 γ for rats as shown by the work of many others. That is, the young rat requires only twice as much B_1 as the mouse, although its weight is five to six times as great. To prevent polyneuritis in the mouse requires about 1 γ daily, while 1 to 2 γ is sufficient for the rat.

In Table XXIII are given the authors' best estimates of B_1 requirement, as taken from the literature, to prevent polyneuritis in the several species. Figures on requirement for promotion of growth are included for the two species for which they are available.

TABLE XXIII

THIAMIN REQUIREMENT (AS CHLORIDE) OF VARIOUS SPECIES

Species	To Promote Normal Growth		To Prevent Polyneuritis		
	γ Thiamin Daily		γ Thiamin Daily		Parts per Million in Food[5]
	Per Animal[1]	Per Kg. Body Wt.	Per Animal[1]	Per Kg. Body Wt.	
Rice bird	—	—	2	66	1
Mouse	2[2]	100	1.0[2]	50	0.5
Rat	4	30	2.0	16	0.4
Pigeon	—	—	9.0[6]	22.5	0.7
Chick	—	—	—	—	0.7[7]
Dog	—	—	64[3]	8[3]	0.35[3]
Man	—	—	600[4]	10[4]	0.60[4]

[1] A typical sized animal: mouse, 20 grams; rat, 125 grams; pigeon, 400 grams; dog, 8 kilos; man, 60 kilos. Of these the mouse and rat are picked as young, half-grown animals because the available data refer to such.

[2] Private communication of Prof. L. R. Cerecedo.

[3] These values are derived from Cowgill's earlier conclusion. Food consumption was taken as 180 gm. per day (R. G. Linton, Vet. Med. *32*, 86 (1937)).

[4] Conclusion reached by analysis of diets associated with outbreaks of beriberi.

[5] This presumes a highly starchy diet except in case of the dog. With high-fat diets the requirement should be less.

[6] Ohdake, S., and Yamagishi, T. (Bull. Agri. Chem. Soc. Japan *11*, 111 (1935)) show cures with 3 γ and protection with 5 γ. Smaller protective doses were not tried. However, the polished rice used was unwashed and must have supplied 4 to 5 γ daily in addition to the crystalline substance given. Spruyt gives values equivalent to 0.5 γ per gram as an average figure for polished rice. Food consumption taken as averaging 10 gm. per day, which is too little for full nutrition but fully as much as pigeons will eat on a bare subsistence basis.

[7] A. Arnold and C. A. Elvehjem, Rochester Meeting, Amer. Chem. Soc., Sept. 8, 1937.

It is evident that the concentration required in the food is of the same order of magnitude for all species considered. The birds, perhaps because they are seed eaters, seem to have a higher requirement than mammals of corresponding size. Data on fish-eating birds and nut-eating squirrels would be of interest, as they would help to throw light on the question of whether the species differences arise from food habits or from the anatomical characteristics of classes or genera. Is the high thiamin content of pork a reflection of an unusually high requirement in the hog or

of a better conservation of thiamin? Is there a connection between the high thiamin content of pork and the hog's tendency to put on fat? If so, would geese show a similar high storage? What is the nature of thiamin economy in hibernating animals such as the bear?

Cattle represent a species which often derives a large part of its supply of thiamin from bacterial synthesis in the alimentary tract (see page 378, Chapter XXVIII). This synthetic activity evidently also gives rise to other B vitamins, but the subject has been studied little chemically and not at all quantitatively.

The reason for having discussed at such length the theoretical portion of Cowgill's presentation is that the theory, if accepted, has some extraordinary implications of profound significance. This theory implies that a maximum normal weight attained by a species represents at least a theoretically attainable weight for every member of that species. The theory also suggests that the species distinctions do not represent merely a man-made classification of convenience, but rest upon some very deep principle of nature. This is true because Cowgill visualizes the B_1 requirement rising rapidly with weight within any species and at the same time falling as weight rises from species to species. If its implications were accepted, the Cowgill theory would have a profound significance with regard to our ideas of the origin and nature of species. In the light of present knowledge, we are accustomed to regard the variations from breed to breed or variety to variety as minor instances of the same sort of variation which we observe in larger degree when comparing one species with another. If, however, the trend of the B_1 requirement remains uniform through a series of breeds and varieties, but abruptly changes in passing from one species to another, then species differences must be not only quantitatively but also qualitatively different in character from breed or variety differences. Each species must then be regarded as a distinct universe in the statistical sense, no matter how diverse the varieties or how great the range of habit or customary size. Our own analysis of the facts permits an interpretation which is much more consistent with generally accepted ideas of the origin of species.

Summary

All species of animals require somewhat less than one part per million of thiamin in the food when carbohydrate predominates in the diet to the degree which is usual for most species. The species differences in this regard are almost, if not quite, within the limits of present experimental error. Large species require less vitamin per unit of body weight than small ones, roughly in proportion to the lesser food intake per unit of body weight

which characterizes the larger species. The differences between species and species in this regard are not of a greater order of magnitude than corresponding size differences among the individuals of a given species. Thus all species of animals which have been extensively studied appear to constitute a rather homogeneous group with regard to thiamin requirement. This justifies faith in the application of experimental data secured from animals to the problem of human requirement.

BIBLIOGRAPHY

1. Cowgill, G. R.: Vitamin B Requirement of Man. Yale University Press, New Haven, 1934.
2. Jansen, B. C. P., and Donath, W. F.: Isolation of the Anti-beriberi Vitamin. Mededeel. van den Dienst. Volksgezondheid. Ned. Ind. *16*, 186, 1927.
3. Freudenberg, W., and Cerecedo, L. R.: Use of Albino Mice as Test Animals for Determining the Potency of Antineuritic Concentrates. Jour. Biol. Chem. *94*, 207, 1931.
4. Cerecedo, L. R., Hennessy, D. J., Kaszuba, F. J., and Thornton, J. J.: Meeting Amer. Chem. Soc., April 13, 1937.

CHAPTER XXV

DISTURBANCE OF GLYCOGEN STORAGE AND BLOOD SUGAR IN THIAMIN DEFICIENCY

As a basis for the discussion of this topic it may be well to restate some of the established elementary facts regarding carbohydrate metabolism in normal animals. Unfortunately these are so uncertain that we soon find ourselves in the realm of the unknown and must tentatively accept as a substitute for facts current ideas which have behind them a weight of well-informed opinion. To state this situation in some detail even at the cost of appearing pedantic seems justified in view of the need for fixing the role of thiamin in the body. That it is intimately concerned in the intermediate carbohydrate metabolism is abundantly indicated by existing evidence, but how and where are still far from clear. There can be little doubt that a full understanding of the function of thiamin in this connection will help to clarify the whole subject of carbohydrate metabolism generally. Indeed such a clarification may well prove to be the major value growing out of researches on this vitamin.

Glucose is the only sugar that remains in normal blood in appreciable amounts. It is derived from carbohydrate or proteins of the food and normally attains a value of about 0.07 per cent in the blood. Only when it passes the renal threshold of about 0.16 per cent does it begin to be excreted in the urine. Large amounts of it may be stored in the liver and the muscles as glycogen, where it constitutes a ready reservoir to be drawn upon as needed for muscular work or heat production. The amount of storage in the liver varies from hour to hour, depending on the amount and recency of absorption of glucose from the digestive tract and the rate of expenditure of glucose for energy production. Glycogenesis and glycogenolysis, controlled by the enzyme glycogenase, are balanced against one another in a remarkably flexible way. The balance, however, is readily disturbed by internal secretions such as adrenalin, by brain injury ("sugar puncture"), and by many variables in the composition of the body fluids. In view of the fact, among others, that inorganic phosphates in the blood fall and phosphate excretion in the urine diminishes as a result of glucose absorption, it is gen-

erally agreed that an early step in glucose absorption is the phosphorylation of the sugar to corresponding esters, a process which also occurs when the sugar undergoes splitting.

Some portion of the glucose may be converted into a secondary energy reserve in the form of fat. It is commonly supposed that pyruvic acid, derived from lactic acid, is an intermediate in this process. Pyruvic acid may be converted in part into acetaldehyde by the enzyme carboxylase, in which case one can picture acetaldehyde condensing with pyruvic acid or with itself by an aldol type of condensation to form long fatty acid chains. The process, whatever its exact mechanism, is presumably reversible so that fat may be reconverted into sugar and utilized in that form.

Glucose is, of course, the most important fuel of the body, but the method of its combustion is still very obscure, in spite of the prodigious increment of knowledge which the discovery of insulin has brought about. The fact that insulin is indispensable to the normal oxidation of sugar is, of course, a commonplace, but its mode of action and its relationship to other oxidative or hydrolytic mechanisms is still extremely obscure. That there is an apparent vague kinship between the function of thiamin and that of insulin adds further importance to our topic.

In addition to the above-mentioned potential destinations for absorbed glucose, we must also consider muscular activity. Muscular contraction is an anaerobic process involving the simultaneous disappearance of muscle glycogen and the appearance of lactic acid, phosphoric acid, and some glucose. It is attended by the production of about twice as much heat as can be accounted for by the conversion of glycogen to lactic acid. A sugar phosphate is commonly supposed to be formed as an intermediate, and a kinship of the chemical processes of muscular contraction to those of fermentation is indicated by a considerable mass of evidence.

During recovery after muscular contraction oxygen is taken up, carbon dioxide is evolved, lactic acid and glucose disappear, and a less than corresponding amount of glycogen is regenerated. In fatigue lactic acid accumulates and a generous supply of oxygen is required to dispose of it, although the primary process of disposal of lactic acid is not an oxidation, but a conversion to glucose. This is shown by the fact that injection of lactic acid into phlorizinized animals results in the excretion of almost the equivalent amount of sugar in the urine.

Before considering further the mechanisms involved in the upbuilding and degradation of sugar in the body, we may well survey the relationship of thiamin to the storage in the body of glucose in its various forms. The literature with reference to the effect of avitaminosis upon glycogen storage is very confusing.

We are indebted to Westenbrink (1) for a critical review of the earlier literature. We will scan this review briefly so that the reader may see first hand the conflicting nature of the evidence.

Funk and von Schonborn (2) forcibly fed two groups of pigeons respectively with 12½ grams each daily of the following basal diets:

	Fat-free Diet Per Cent	*Sugar-free Diet Per Cent*
Casein	12	12
Sugar	42	—
Starch	42	42
Salts	4	4
Fat	—	42

Some of the birds on each diet received an addendum of yeast. The authors reported results as follows:

Diet	*No. of Days Fed*	*No. of Birds*	*Per Cent Glycogen in Liver*
Fat free	14	5	5.6; 3.6; 9.4; 0.9; 2.2
Fat free plus yeast	14	1	0.4
Sugar free	12–14	6	0; 0; 0.01; 0; 0; 0
Sugar free plus yeast	12–13	3	0.7; 0.5; 0.7

The figures for the liver glycogen content of the birds fed upon the fat-free diet alone cover a wide range but appear to indicate normal glycogen or even an increase of liver glycogen above normal in B avitaminosis. By contrast, the birds fed on the sugar-free diet appear to have been depleted of liver glycogen as a result of the deficiency of B vitamins. Later one of these authors, Funk (3), fed pigeons for twelve days with 30 grams of polished rice daily and found the following liver glycogen values: 5.49; 4.05; 1.72; 5.67; and 3.51.

Collazo (4) experimented with pigeons, chickens, and dogs. He does not state the number of animals used, and only average glycogen values are given. In normally fed chickens and pigeons he found 2.1 per cent and 3.1 per cent of liver glycogen respectively; on a diet of polished rice the respective values for the two species were 0.1 and trace; for starved birds 0.5 and 0.15. Dogs also were used, the diet consisting of 50 grams of vitamin B-free horseflesh, 50 grams of polished rice, 30 grams lard, and 3 grams salts. The normally fed dogs showed 5 per cent liver glycogen; the B-free animals 0; and the starved 1 per cent. Collazo's results appear to indicate a reduction in liver glycogen in avitaminosis as well as in starvation. However, Westenbrink thinks it is possible that starvation is the cause of the lowering of glycogen in both instances, since the digestive tract was found full of food at death, indicating an impairment of assimilation.

Recognizing that glycogen storage varies from hour to hour, depending on the rate of absorption of glucose from the ali-

mentary tract, Rubino and Collazo (5) introduced a different technique in studying the subject. They administered 5 grams of glucose to a series of birds in normal and in a more or less avitaminotic condition. After intervals of two, four, and six hours, respectively, pairs of the birds were killed and glycogen was determined in the livers. The object clearly was to ascertain the rate at which glucose is converted into glycogen, as well as the rate at which the liver glycogen is subsequently utilized.

GLYCOGEN PER CENT

	2 HOURS	4 HOURS	6 HOURS
Normal	5.93	7.12	6.85
	1.11	5.00	5.69
Fed two weeks on polished rice	10.0	4.1	—
Fed three weeks on polished rice	2.85	1.2	—
Fed four weeks on polished rice	0.20	0.09	—

In addition, pigeons were fed for twenty-five to thirty-one days on polished rice; 10 grams of each of the following sugars were then administered to individual birds, and four hours later liver glycogen determinations were made, with the results indicated below:

Glucose	5.4 and 3.1 per cent	Maltose	2.0 and 1.8 per cent
Fructose	2.0 and 2.0 per cent	Lactose	1.0 and 1.0 per cent
Galactose	1.0 and 1.0 per cent	Starch	0.6 and 0.5 per cent

Additional similar results were obtained on other pigeons in the fourth week of avitaminosis. The conclusion is drawn by the authors that the glycogen-forming power and glycogen utilization are not impaired.

Randoin and Lelesz (6) fed 27 pigeons on a normal diet (A); 27 on a synthetic diet with yeast addition (B); and 36 on essentially the same diet without yeast (C). The birds on the synthetic diets were fed artificially with 20 grams of food daily. At intervals several birds were killed and the glycogen was determined in a composite sample composed in each case of the livers of three pigeons. The birds killed at the end of period I showed no certain symptoms of illness; at the end of period II the birds on diet C which were killed exhibited lowered body temperature and incipient paralysis; those at the end of period III were severely paralyzed. The results did not give any indication of impaired glycogen formation (page 320).

Collazo and Pi-Suñer Bayo (7) conducted similar studies. For five normal pigeons they found an average of 3.039 liver glycogen; for five pigeons on a B-free diet 0.129; for five birds fed twenty days on a B-free diet and then given daily 1 c.c. of yeast autolysate for five days, 6.423 per cent liver glycogen. The

LIVER GLYCOGEN PER CENT

PERIOD	C	B	A
I	2.81	2.86	3.79
	2.50	2.88	3.12
	3.23	2.88	3.12
	2.92	3.27	2.90
	0.56	2.44	2.90
	2.75		
	2.75		
II	1.38	5.04	3.97
	2.46	5.04	3.97
	0.38		
III	3.32	4.70	3.95
	3.56	4.70	

birds apparently were not artificially fed, and the indicated glycogen depletion in B-deficient birds may have been due to inanition.

In the later studies these authors made comparisons of the effect of heated and unheated yeast autolysate, and of heated and unheated orange juice as addenda to an otherwise B-free pigeon diet. In view of the fact that the yeast autolysate and orange juice were heated only to 100 degrees for short periods of time, there is no reason to suppose that any considerable fraction of the B_1 which they contained was destroyed as the authors evidently intended. Nevertheless, the authors purported to find an impairment of glycogen formation in the liver in this mild avitaminosis.

Later and more elaborate studies on pigeons have been conducted by Abderhalden and Wertheimer (8, 9). According to the findings presented in the first paper birds fed on a B_1 free diet show abnormally high liver glycogen.

a. Pigeons in paralytic condition, having suffered an average weight loss of 24 per cent, showed liver glycogen contents as follows: 8.0; 8.3; 1.12; 1.36; 6.1; 9.2; 12.0.

b. Birds in the early stages of rice feeding, having suffered only a 5 per cent weight loss, had the following liver glycogen contents: 1.15; 1.0; 0.06; 2.1; 0.50; 0.77; 0.29.

c. Pigeons in the later stages of avitaminosis but without paralysis and with a weight loss of 29 per cent had the following liver glycogen contents: 6.7; 10.4; 8.2; 1.7; 3.2; 6.4.

d. Starved pigeons with a weight loss of 19 per cent had only the following per cent glycogen: 0.01; 0.025; 0.020; 2.10; 0.018.

e. Birds which had become paralyzed were found, after administration of yeast without other food, to contain the following percentages of glycogen in their livers: 4.2; 0.017; 7.2; 0.002; 0.33; 0.0008; 6.0; 0.016.

f. Paralyzed pigeons subsequently treated in the same way

with autoclaved yeast showed liver glycogen contents of: 8.5; 1.74; 0.072; 10.0; 1.0.

In the second of these papers the authors reaffirmed their previous ideas. They believe that liver glycogen is supernormal in avitaminosis and that the administration of B_1 not only dissipates the paralytic symptoms but also reduces liver glycogen. In the series of experiments described in this paper diets were used in which the proportion of carbohydrate, protein, and fat were varied over wide ranges. As a result they conclude that the effect of protein in the diet in the absence of B_1 is like that of carbohydrate, namely, to bring about glycogen formation in the liver, and that the so-called sparing action of fat is not a true sparing action but simply results from the fact that less glycogen is formed from fat than from either carbohydrate or protein.

It is not possible to summarize all the foregoing experiments on pigeons so as to develop a consistent thesis. Many of the experimental difficulties doubtless arise from the fact that the liver glycogen varies widely in normal animals, from individual to individual, and from hour to hour, depending on the time and extent of feeding and the rapidity of assimilation. These facts make it almost impossible to establish a norm with any degree of precision. In addition, in B_1 avitaminosis there is marked impairment of appetite and of the ability to assimilate food, so that whether artificial feeding is practiced or not, polyneuritis is always complicated by a greater or lesser degree of inanition. This makes it extremely difficult to differentiate between the effects of B avitaminosis *per se* and the secondary effects of partial starvation. On the whole, the results of Abderhalden and Wertheimer on pigeons are more numerous, more consistent, and more impressive than those of other workers in the field. It is especially noteworthy that their work includes a few experiments with crystalline B_1 (oryzanin), in which the probability of effects due to extraneous substances, notably other B factors, is very greatly reduced. These results appear to justify the very tentative conclusion that in acute polyneuritis in pigeons uncomplicated by inanition there is supernormal glycogen storage and that the administration of B_1 to such birds accelerates glycogen utilization and brings about a decline in liver glycogen.

We may now turn to studies of the subject which have been conducted upon rats; the first of these is by Eggleton and Gross (9), who fed rats of 100 to 150 grams' weight on a diet consisting of 100 grams rice starch, 40 grams casein, 20 grams butter, 10 c.c. lemon juice, 10 grams salts. Control animals were given the same diet with an addition of 10 grams of yeast extract. The authors found a very much lower glycogen content in the livers of avitaminotic rats, but this was probably due to inanition, as the animals were reported to be "almost in a fasting condition at the end." However, by killing animals at intervals in the course

of such feeding on the B-free diet, the authors concluded that a drop in liver glycogen occurs more often in the first ten days of avitaminosis. This would indicate a genuine impairment of glycogen storage, but the conclusion is not supported by details as to the number of rats used or the individual glycogen values found.

Westenbrink's experimental results with which he concludes the above-mentioned review (*loc. cit.*) are reported in very much more satisfactory detail. Groups of rats between 150 and 200 grams in weight were fed for five to eight weeks upon the following diets:

(A) Normal
- 500 parts of unbolted wheat meal
- 1000 parts of whole milk powder
- 5 parts of cod liver oil

(B) B_1 Deficient
- 900 parts washed rice meal
- 30 parts casein
- 30 parts salt mixture
- 100 parts autoclaved yeast
- 10 parts cod liver oil

(C) Thermostable B Deficient
- Same as diet D with 0.3 per cent activated acid clay

(D) B Complex Deficient
- 100 parts casein
- 100 parts rice starch
- 40 parts olive oil
- 50 parts salt mixture
- 5 parts cod liver oil

All rats were fasted for twenty-four hours, after which all but a few controls were given by mouth 2 c.c. each of 50 per cent glucose solution and killed four hours later.

Liver Glycogen Per Cent

No. of Rats	Diet	Glucose	Glycogen
4	A	None	0.3; 0.2; 0.2; 0.2
2	B [1]	None	0.2; 0.2
18	A	2 c.c.	2.0; 0.8; 1.8; 0.5; 2.9; 1.4; 1.6; 2.2; 0.9; 1.4; 1.3; 2.2; 0.6; 1.7; 3.0; 2.3; 2.0; 1.6
13	B [1]	2 c.c.	1.3; 1.0; 0.3; 1.7; 1.7; 1.4; 2.1; 1.6; 2.2; 1.6; 0.8; 2.8; 1.9
15	C [1]	2 c.c.	1.6; 1.3; 0.5; 2.9; 2.2; 2.2; 2.2; 1.2; 1.2; 1.0; 0.6; 0.9; 2.0; 1.6; 2.0
13 [2]	D	2 c.c.	2.2; 2.0; 3.9; 3.9; 2.8; 3.5
6 [3]	D	None	0.3; 1.6; 0.5; 0.2; 0.2; 0.3
10 [3]	D	2 c.c.	2.6; 0.2; 3.6; 0.4; 4.2; 3.3; 2.7

[1] Without paralysis but judged to be within two or three days of death.
[2] Some rats died during fasting.
[3] Not fasted.

A comparison of all the above results indicates no difference between the normal rats and those in various stages of avitaminosis, four hours after administration of glucose.

Further experiments on normal, fasted rats at intervals of two, six, and eight hours after administration of glucose gave the following results:

2 Hours	*6 Hours*	*8 Hours*
1.9	2.0	0.4
1.5	1.1	0.4
1.3	0.5	0.3
1.3	1.4	0.3

Rats fed on diet D to approximately 40 per cent weight loss prior to fasting and glucose gave these values:

2 Hours	*6 Hours*	*8 Hours*
0.3	2.3	0.3
0.3	1.2	0.3
1.8	1.7	
1.0	1.1	

As regards rats, the literature conflict is not serious, since the experiments of Eggleton and Gross admittedly involved inanition. Westenbrink seems to be justified in concluding from his rather extensive experiments that glycogen is fairly normal in the liver of rats in various stages of B avitaminosis.

The conclusion that liver glycogen is sometimes high in polyneuritis in pigeons but usually normal in B avitaminosis in rats is confirmed by other work not covered in Westenbrink's review. Schrader (10) reports a frequent occurrence of very high liver glycogen as well as high blood sugar in an advanced state of beriberi, while rats appeared substantially normal. These papers, while very brief and lacking in detail as presented, appear to cover an exceptionally thorough study with adequate numbers of animals and good controls. As the papers are probably not widely accessible, it seems well to review them in some detail. In the first paper an account is given of glycogen determinations on 200 rats and 30 pigeons, also 200 blood sugars on rats and 100 on pigeons. The animals were fed on a diet containing 77 per cent sucrose and, presumably, casein and salts. Autoclaved yeast was supplied to all. Vitamin B_1 was given to the control groups in the form of fuller's earth adsorbate. Controls on "normal" diet were also used for comparisons. The animals were starved for twenty-four hours before administration of glucose or lactic acid and killed four hours afterward. Preliminary experiments showed that 125 to 500 mg. of glucose per rat was the best level for significant results. Amounts of lactic acid in excess of 250 mg. per rat were found harmful for normal as well as for B-deficient rats, even though half neutralized with sodium hydroxide.

Lactic acid and glucose were given by mouth. In certain cases insulin was administered subcutaneously. Separate determinations were made of liver glycogen and body glycogen, *i.e.*, on the entire body minus the alimentary tract and liver.

In the case of pigeons similar methods were used except that 10 to 15 grams of breast muscle, rather than the entire body, were analyzed for glycogen. No specific data are presented in this paper, but the following observations were noted:

(1) Beriberi was usually accompanied by a high blood sugar in the pigeon, especially during the final stages before death. In some cases a value twice that of the normal bird was observed. In the rat there was little, if any, increase in the blood sugar. This discrepancy can possibly be explained in that the pigeons were force-fed, whereas the rats were not. The rats consumed very little food, especially during the last week to ten days.

(2) Beriberi pigeons appeared to be more susceptible to insulin shock than did normal birds. This was found to be due, in part at least, to the power of the insulin to lower the blood sugar. The symptoms of insulin shock in the vitamin B-deficient bird resembled the actual polyneuritic symptoms. Insulin shock was apparently produced at a higher blood sugar level in the vitamin B-deficient bird than in the normal. However, the simultaneous administration of glucose and insulin prevented such a pronounced decrease in the blood sugar and likewise prevented insulin shock. It thus appeared that the vitamin B-deficient bird could still adsorb glucose from the alimentary tract. No similar work has been done on the rat as yet.

(3) The cure of beriberi in the pigeon by the administration of brewers' yeast was accompanied by a return of the blood sugar to normal values in from two to three days.

(4) Starvation caused a depletion of liver and body glycogen in the vitamin B-deficient rat as well as in the normal rat. In 24 hours the liver glycogen was reduced to practically zero in both cases.

(5) Both d-glucose and r-lactic acid (one-half neutralized with NaOH) induced glycogen formation in vitamin B-deficient as well as normal rats, although quantitative differences may exist.

(6) In cases where large amounts of glucose or r-lactic acid were administered to rats, death often resulted. This was especially true for the higher lactic acid levels. Upon autopsy the stomachs and small intestines were found to be decidedly hemorrhaged. If death did not occur, very high blood sugars were found. This again showed that the materials were absorbed, and in the case of lactic acid apparently converted into blood sugar.

(7) Pigeons with advanced beriberi, that is, the convulsive stage, often were found to have a very high liver glycogen, as well as the high blood sugar previously mentioned.

In conclusion we can only say that the major part of the evidence thus far obtained indicates no serious defect in the glycogen formation or glycogen liberation stages in the vitamin B-deficient animal. The evidence, however, is not yet convincing. The fact that the pigeon and rat results have not always been in harmony may be due to: (1) The pigeons are force-fed, whereas the rats are not, and (2) the only ration used in the rat work to date has been the high sucrose ration 3-G upon which the rats usually die before exhibiting actual polyneuritis. We are at present trying to develop a ration which will consistently produce actual beriberi in the rat.

The 1933 paper deals with the respiratory metabolism during thirty-minute periods four hours after giving glycogen or lactic acid to (*a*) beriberic rats, (*b*) B_1 deficient rats showing no symptoms, and (*c*) rats on B_1 adequate diets. Heat production was raised 17, 9, and 15 per cent, respectively, for the three groups on receiving 125 mg. of glucose per animal. There was also a rise in respiratory quotient in all three groups. The glucose administered was converted into glycogen to the extent of 53, 72, and 54 per cent, respectively, for the three groups.

In the 1934 paper comparisons are made of the glycogen contents of starved versus unstarved rats which had been fed on one of two B_1 deficient diets and also of rats which had been fed on one of two B_1 adequate diets.

THE QUANTITY OF GLYCOGEN IN THE VITAMIN B-DEFICIENT RAT AND ITS ABILITY TO DEPLETE THIS GLYCOGEN DURING STARVATION

DIET	NUMBER OF RATS	TREATMENT	GLYCOGEN PER 50-GM. RAT (MG.)	DIFFERENCE (MG.)
3 G *	13	Starved	78.6	—
	17	Not starved	223.9	145.3
552 †	47	Starved	112.4	—
	21	Not starved	195.1	82.7
552 plus vitamin B	9	Starved	110.4	—
	13	Not starved	225.1	114.7
Stock	26	Starved	113.5	—
	3	Not starved	324.8	211.3

* Diet 3 G contained 77 per cent of sucrose.
† Diet 552 contained 43.4 per cent of sucrose and 23 per cent of fat.

Evidently the capacity of B_1 deficient rats to store glycogen in the liver is little impaired considering that the B_1 deficient rats eat less food, nor do the B_1 deficient animals lose the capacity to utilize stores of liver glycogen during starvation. It is regrettable that the original experimental data on individual animals are lacking in Schrader's reports.

Lajos (11) conducted a series of experiments involving large

numbers of animals in an apparently thoroughgoing manner. He administered glucose to rats that had been starved for four days and slaughtered them at intervals of time ranging from 20 to 240 minutes. When the glucose was accompanied by insulin, the rise of liver glycogen and muscle glycogen was much more marked than without insulin. The rise in blood sugar following administration of glucose was markedly less when insulin was given with the glucose. The reported action of B_1, as administered with glucose, was similar in character and equally marked in all three respects but was slower than that of insulin. An apparently fatal defect in the experiments was that the B_1 used was the International Standard adsorbate. Fifty mg. of this, per 100 gm. weight of animal, was suspended for twenty-four hours in 5 c.c. physiological salt solution and then filtered off. To the filtrate the appropriate amount of glucose was added and the solution was injected into the peritoneum. According to all our experience, under these circumstances the vitamin B_1 would remain adsorbed on the acid clay and would be discarded with it. Two possible explanations occurred to us. Either the observed action of the extract was due to something else than B_1 or the sodium chloride displaced the vitamin from the clay by a permutit type of action. That this is possible, though not probable, was suggested by the fact that Cerecedo (12) has reported the adsorption of B_1 by artificial zeolite and its elution by sodium chloride. We tried this expedient with fuller's earth adsorbate many years ago without success. Messrs. R. E. Waterman and W. L. Sampson have kindly studied the elution of the vitamin from the International adsorbate, and communicated their results to us. Even four times the amount of adsorbate which Lajos used in his experiments does not yield enough thiamin by sodium chloride elution to cure polyneuritis in rats. We must conclude that Lajos' results were due to some other substance than thiamin.

In this connection mention should be made of some evidence of a liver substance controlling glycogen storage. A deficiency of this substance has been associated with B_1 avitaminosis. Briganti (13) states that the substance is capable of reducing blood sugar level, especially in diabetics and depancreatized dogs, as well as to some extent in normals. It seems possible that this is related to the substance which is associated by the Japanese workers with B_1 deficiencies which they refer to as Yakriton. Many papers in the *Tohoku Journal of Experimental Medicine* refer to it, *e.g.*, that of Asakura (14).

Similarly conflicting data could be cited as to the effect of B_1 deficiency on blood sugar. Indeed many of the authors, notably Abderhalden and Wertheimer, above cited, dealt with blood sugar as well as glycogen in their experiments, but the results are even more difficult to appraise. A few other papers

should be mentioned. Bell (15) noted a marked rise in "true blood sugar" during polyneuritic convulsions in pigeons but not in rats. This author believes that hyperglycemia in pigeons is caused by nervous stimulation. In rats depletion of glycogen is regarded as preventing a rise of blood sugar. Indeed Bell notes frequent occurrence of hypoglycemia in the last stages of polyneuritis of rats.

Rats and pigeons differ markedly as B_1 test animals. The latter are more erratic; the time required to develop polyneuritis varies more widely from individual to individual, the proportion of animals which develop characteristic symptoms is less constant, and the onset of the symptoms is much more sudden and intermittent. Spontaneous recovery is unknown in rats but common in pigeons. It seems very probable that there is a connection between the differences in glycogen metabolism and these contrasts in external behavior.

It is perhaps of interest that Corkill (16) has found marked contrasts among normal animals of several species (rabbit, chicken, ferret, and mouse) with respect to the effect of insulin on the glycogen contents of liver and muscles. For example, insulin causes an accumulation of liver glycogen in the rabbit, prevents deposition of glycogen in the liver of chickens, has no effect on that of the ferret, and causes a rapid discharge in the case of the mouse.

As further evidence of the confusion we may well cite Lewinson (17), who, in one of the most recent articles on the subject, summarizes his conclusions somewhat as follows:

1. A B_1 deficiency leads to a disturbance of carbohydrate metabolism quite apart from that caused by inanition.
2. This carbohydrate disturbance precedes the nervous symptoms.
3. The blood sugar curve on administration of glucose rises abnormally rapidly and subsequently sinks more slowly than in normal animals.
4. In the course of the development of polyneuritis in dogs the blood sugar falls in the early stages and later rises. In pigeons hyperglycemia predominates throughout.
5. Lactic acid in blood rises in all common experimental species as B_1 deficiency develops.
6. Ketone bodies, especially oxybutyric acid, also rise in the blood, but this may be a secondary consequence.
7. Liver glycogen falls in both dogs and pigeons in B_1 deficiency.
8. The effect of injection of glucose on the blood sugar curve and the determination of lactic acid in the blood are proposed as diagnostic tests.
9. The disturbance of carbohydrate metabolism is extremely complicated and is related to nerve damage.

Lewinson fails to give us detailed experimental data in support of many of his conclusions.

We detect a hopeful gleam of light in a more recent paper (18) by Martin. He fed depancreatized dogs on a diet almost free of both thiamin and riboflavin. Despite regular and abundant injections of insulin, the animals developed very marked hyperglycemia and glycosuria in the course of six to seven weeks, and death followed very soon. Increase of insulin dosage up to eight times the normal only temporarily and partially rectified the sugar disturbance. However, administration of pure thiamin together with pure flavin corrected it promptly when given with a normal supply of insulin. If the insulin was kept at the high level, the administration of the two vitamins induced hypoglycemic shock. Thiamin alone did not supplement the action of insulin detectably. Transfer of the dogs to normal diets, with regular injections of insulin, gradually effected the same cure of the sugar disturbance as that observed on giving the two vitamins.

We consider this paper so suggestive that if its findings are verified, it should reopen the whole question of thiamin deficiency in relation to sugar disturbances. It seems plausible to suppose that a necessity for action of the two vitamins in concert could account for all, or nearly all, the discrepancies in the literature, especially the discrepancies in the behavior of the rat and the pigeon. The former has such a marked need for riboflavin that it has become universal practice to include it, or a good source of it, such as autoclaved yeast, in experimental diets for rats. Pigeons, however, reveal so little need of it that it is quite customary to omit autoclaved yeast or its equivalent from their diets.

Summary of Conclusions

There is convincing evidence of frequent disturbance of glycogen storage and blood sugar in B_1 avitaminosis. Further indications of this are found in clinical experience (Chapter IV, page 72). However, the direction and extent of the deviations from normal are such as to make it improbable that there is a specific, uncomplicated, and primary relationship between thiamin and these functions. Marked irregularities in the experimental evidence appear to be associated in part with species differences, in part with the complicating effects of attendant inanition or subsidiary deficiencies, and in part with variations in the character of damage to the central nervous system. A dual deficiency of thiamin and flavin is suggested as the most probable cause of the disturbance.

BIBLIOGRAPHY

1. Westenbrink, H. G. K.: Vitamin B und Leberglykogen. Arch. Neerl, Physiol. *18*, 429, 1933.
2. Funk, C., and von Schonborn, E.: Influence of a Vitamin Free Diet on the Carbohydrate Metabolism. Jour. Physiol. *48*, 328, 1914.
3. Funk, C.: Action of a Substance Influencing Carbohydrate Metabolism in Experimental Beriberi. Jour. Physiol. *53*, 247, 1919.
4. Collazo, J. A.: Der Stoffwechsel bei Avitaminose. II. Glycogen und Avitaminose. Biochem. Zeit. *136*, 20, 1923.
5. Rubino, P., and Collazo, J. A.: Untersuchungen über den intermediären Kohlenhydratstoffwechsel bei Avitaminose. I. Glykogenbildung und Umsatz bei der Avitaminose. Biochem. Zeit. *140*, 258, 1923.
6. Randoin, L., and Lelesz, E.: Variations Comparatives de la Glycémie Arterielle et de la Teneur du Foie en Glycogène chez le Pigeon Normal et chez le Pigeon Soumis à un Régime Desequilibré par Manque du Facteur Hydrosoluble B. Compt. Rend. *180*, 1366, 1925.
7. Collazo, J. A., and Pi-Suñer Bayo, P.: Über die Wirkung der B-Vitamine und des Insulins auf die Kohlenhydratstoffwechselstörungen bei Mangel an B-Vitaminen. Biochem. Zeit. *238*, 335, 1931.
   Einfluss des Vitamins B_2 auf den Kohlenhydratstoffwechsel. Biochem. Zeit. *250*, 89, 1932.
8. Abderhalden, E., and Wertheimer, E.: Über das Verhalten des Glykogens im Organismus bei Abwesenheit des Vitamin B Komplexes (insbesondere des Vitamin B_1) in der Nahrung. Arch. ges. Physiol. *230*, 601, 1932.
   Beziehungen des Vitamin-B-Komplexes (insbesondere des Vitamin B_1) zum Kohlehydrathaushalt. Arch. ges. Physiol. *233*, 395, 1933.
9. Eggleton, P., and Gross, L.: Note on the Blood-sugar Level of Rats Fed with Complete and Diets Deficient in Vitamin B. Biochem. J. *19*, 633, 1925.
10. Schrader, G. A.: Relation of Vitamin B to Carbohydrate Metabolism. Ala. Agr. Exp. Sta. Annual Rept. *42*, 35, 1931.
    Ability of the Vitamin B-deficient Rat to Utilize d-glucose. Ala. Agri. Exp. Sta. Annual Rept. *44*, 20, 1933.
    Quantity of Glycogen in the Vitamin B-deficient Rat and Ability to Deplete This Glycogen during Starvation. Ala. Agri. Exp. Sta. Annual Report. *45*, 21, 1934.
11. Lajos, S.: Über die Wirkung des B-Vitamins auf den Kohlenhydratstoffwechsel. Biochem. Zeit. *284*, 279, 1936.

12. Cerecedo, L. R., and Hennessy, D. J.: On the Use of Synthetic Zeolites in the Isolation of Vitamin B_1. Jour. Amer. Chem. Soc. *59,* 1617, 1937.
13. Briganti, A.: Hypoglycaemic Action of Liver Extract. Riv. Patol. Sper. *16,* 469, 1936.
14. Asakura, K.: The Detoxicating Hormone of the Liver (Yakriton). Tohoku Jour. Exp. Med. *19,* 269, 1932.
15. Bell, M. E.: Blood Sugar Level in Vitamin B_1 Deficiency. Biochem. J. *25,* 1755, 1931.
16. Corkill, B.: The Influence of Insulin on the Distribution of Glycogen in Normal Animals. Biochem. J. *24,* 779, 1930.
17. Lewinson, M. S.: Dynamik des Kohlehydratstoffwechsels bei Hunden und Tauben in Avitaminose B. Zeit. Vitaminforsch. *6,* 209, 1937.
18. Martin, R. W.: Vitaminfreie Ernahrung und Insulinwirksamkeit. Zeit. Physiol. Chem. *248,* 242, 1937.

CHAPTER XXVI

THIAMIN IN INTERMEDIATE CARBOHYDRATE METABOLISM

In various parts of this book there are set forth reasons for questioning the specificity of the action of thiamin with respect to one body function or another. Thus in Chapter XVII we have questioned the specificity of several of the methods of assay on the ground that they have not been demonstrated to be unaffected by unknown factors. In Chapters XXII and XXIII we have thrown doubt on the evidence of specificity of standards of thiamin requirement and on a specific relationship of thiamin to reproduction, lactation, growth, appetite, or individual weight. At the same time we have set forth what appears to us to be compelling evidence that thiamin profoundly affects all these functions. It is merely the *specificity* of the action of thiamin which is denied, not its importance in relation to these matters.

In Chapter XXIV we have attempted to show that all species of animals are fundamentally alike in their thiamin requirements, the differences being associated with dietary habits, not with fundamental differences in body economy. The feature of dietary habit which is believed to be responsible for these minor species variations is the proportion of carbohydrate, fat, and protein present in the customary food. Likewise in Chapter XXV there is outlined the evidence against the idea that thiamin alone has specifically to do with controlling the storage or utilization of glycogen in the liver or the blood sugar level, though abundant evidence is available that these features of carbohydrate metabolism are frequently markedly disturbed in thiamin deficiency.

Furthermore, in discussing the anatomical changes which occur in beriberi and other less pronounced thiamin deficiencies (Chapters II and IV), emphasis has been given to the widespread character of the changes, extending in greater or less degree to all the organs of the body. The great diversity of therapeutic uses for which thiamin has been recommended, not without substantial experimental evidence in many cases, also reflects a widespread utilization of it, as does likewise the distribution of the substance in the various tissues and in various foodstuffs. Its role in lower forms of life is emphasized in Chapter XXVIII with especial reference to plants and microorganisms.

Thus thiamin appears to be good for everything and at the same time good for nothing in particular. We are put in the position of appearing hypercritical of the conclusions of competent workers on many points to the extent that we fear our criticism may appear wanton. At the same time we run the risk of being charged with credulity and with recommending thiamin as a panacea for all human ills in calling favorable attention to the diverse character of therapeutic uses to which it has been put and advocating its trial in still other disorders.

Whence comes all this specious evidence of specificity on the one hand and of panacean character on the other? The answer to this question is to be found in the present chapter and lies in the fact that thiamin plays a fundamental role in intermediate carbohydrate metabolism. Unfortunately this chapter cannot be writen completely at present, as the chemistry of the reactions involved is by no means fully elucidated. However, enough is known to afford great confidence that further work will only serve to fill in the detail of a picture the general outlines of which are already clear.

Our knowledge of intermediate carbohydrate metabolism is as yet very incompletely developed, but incomplete as it is, it already provides the most significant revelation for which modern biochemistry can claim responsibility. The outstanding fact is that the metabolic processes concerned take place, not in individual organs as a whole or in specialized tissues, but in the cells themselves. No matter how specialized the tissue, no matter what function an organ may serve in the economy of that complex society of cells which constitutes a higher animal or plant, still it carries on its own intermediate carbohydrate metabolism, burning a portion of its food to supply energy needed for synthesis and utilizing the remainder of the food for that synthesis. Just as in a complex modern society external human relationships involve a most intricate exchange of specialized goods and services, so the liver, the brain, the kidney, and all the other organs perform functions not peculiar to their own needs but for the general good, an idea which has been familiar at least since the time of St. Paul. Another correlative analogy which neither has been so long known nor is so well appreciated is that just as each individual in a human society must eat his own food and keep his own home fires burning, so every cell regardless of its function in the society of cells contains its own kitchen and heating system.

Moreover, just as all men, rich or poor, white or black, digest their food by the agency of the same secretions in the alimentary tract, so all cells, we are led to believe, carry on fundamentally the same process of intermediate carbohydrate metabolism. While there are important differences of detail or of relative preponderance of one chemical reaction compared with another

between the human brain cell, for example, on the one hand, and the cell of yeast or staphylococcus on the other, the striking fact is that the fundamental mechanisms are common to all cells, according to our best belief. That thiamin constitutes a feature of one of these fundamental mechanisms is becoming more and more certain in recent months. This goes far to explain why it appears specific for no organ or function of the body but important for all. A further perspective of this far-reaching concept is found in Chapter XXVIII on the role of thiamin in lower forms of life. That some degree of thiamin deficiency should be so common in human beings is due not only to the artificiality of dietary habits, but also to the fact that the body is not thrifty with its thiamin supply (Chapter XIX).

There is abundant evidence that carbohydrate constitutes the principal fuel of cells although it may reach the cell only after undergoing transformation to intermediates. Also there are convincing observations to the effect that carbohydrate metabolism is fundamental to all metabolism. The idea that the developing embryo re-enacts the steps of the evolutionary process of the species is very familiar and is widely accepted as regards the histological changes which are observed. The recapitulation of evolutionary history is not limited to anatomical features but also appears to extend to chemical ones. In the embryo it is carbohydrate metabolism which is first observed; protein and finally fat metabolism begin only in substantially later stages of development.

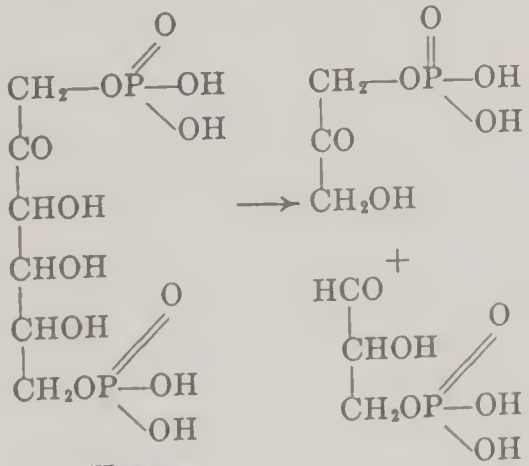
One can explain the varied therapeutic utility of thiamin only on the ground that it is an essential for the metabolism of all cells. A deficiency of it may produce conspicuous defect in whatever organ or tissue happens, in a given individual, to be congenitally subnormal in function or to have been impaired by past disease or existing unfavorable circumstances which may be causing overstrain of its resources. By the same token thiamin cannot, according to this view, be regarded as a "cure" for diseases other than beriberi but merely as an ameliorative agent, the value of which will depend in any given case on the extent to which the previously existing supply of thiamin to the tissue in question has been suboptimal. It is a historical fact which should give us confidence that the groundwork of this "explanation" began to develop before it was known or generally believed that thiamin had a diverse therapeutic utility or that there was a phenomenon of this sort requiring explanation. It originated in tissue respiration experiments directed at purely theoretical objectives, not in clinics where empirical trials of a variety of therapeutic agents are customarily carried out. It was, in fact, a matter of utter surprise to those who had been engaged in the study of beriberi for two or three decades that it should turn out

that what we once called the "beriberi preventing substance" is actually important therapeutically in geographical areas where beriberi is almost unknown. A proper skepticism will, of course, make due allowance for the psychological element in human experimentation and disregard all subjective evidence of benefit from thiamin therapy unless and until this psychological element is eliminated by administration to parallel control individuals of inactive preparations of similar appearance. However, repeated report of such subjective benefits should be given weight to the extent of justifying further open-minded trial, preferably under rigidly controlled conditions.

Carbohydrate Metabolism as a Stepwise Process

Reference has been made in the previous chapter to the interconversion in the body of lactic acid and glucose. The process whereby this conversion takes place is complicated and as yet somewhat uncertain. According to Embden's ideas the process in muscle involves:

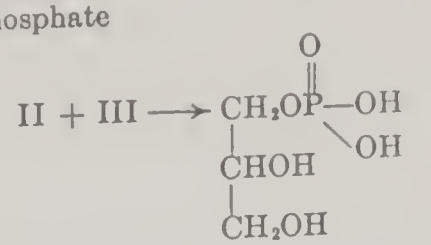
1. Conversion of the hexose to a diphosphoric ester, I (see below).
2. Hydrolysis of the latter to form two triose phosphoric acids, II and III.
3. II and III react, the former being reduced, the latter oxidized by dismutation, a sort of Cannizzaro reaction, to form glycerophosphoric, IV, and phosphoglyceric, V, acids, respectively.
4. V splits to form pyruvic acid, VI, and phosphoric acid.
5. VI is reduced by IV to form lactic acid, VII, and a molecule of III (which can now reparticipate in reaction 3):



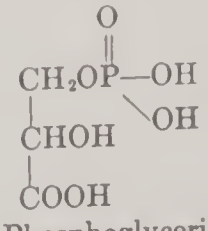
I. Hexose Diphosphate

II. Dioxyacetone Phosphoric Acid

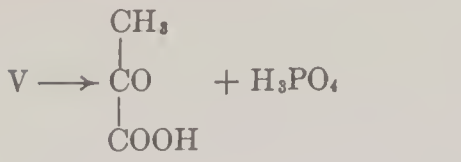
III. Glyceric Aldehyde Phosphoric Acid



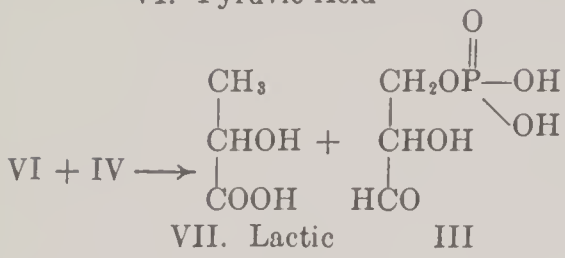
IV. Glycerophosphoric



V. Phosphoglyceric



VI. Pyruvic Acid



VII. Lactic III

II and III above are regarded interconvertible by intramolecular oxidation and reduction.

Each of these steps is controlled by an enzyme of more or less specific character. Each is regarded as reversible, the enzyme functioning to promote the transformation in either direction to equilibrium. Which direction prevails at any moment is determined by the respective concentrations of reactant and reaction products, etc. Each of the enzymes is subject to adverse influences, such as poisons, which may retard or even inhibit the reaction altogether.

Meyerhof has interpreted alcoholic fermentation as an essentially similar series of steps involving in addition the intermediate acetaldehyde and the end product, alcohol:

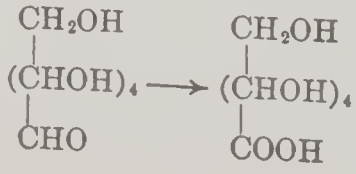
(*a*) Glucose $\longrightarrow$ glucose diphosphate $\longrightarrow$ glycerophosphate + phosphoglycerate

(*b*) Phosphoglycerate $\longrightarrow$ pyruvate $\longrightarrow$ acetaldehyde (CH_3CHO)

(*c*) $2\,CH_3CHO + 1$ glucose $\xrightarrow[\text{acid}]{\text{phosphoric}}$ $2\,C_2H_5OH + 2$ phosphoglycerate

Again a series of specific enzymes, some of them common to the lactate process above described, is in control of the reactions.

In addition to its conversion to lactic acid, glucose is, of course, subject in some very incompletely known manner to oxidation for energy production. This oxidation, as well as the oxidation of amino acids, fats, etc., is controlled by other appropriate systems of enzymes which may be grouped as oxidases. Some of these are anaerobic oxidases, also called dehydrogenases, which act by conferring reducing power on substances which are not otherwise readily reduced, *e.g.*, succinic, malic, and lactic acids. An example which might seem to be of possible interest to us is glucose dehydrogenase, which converts d-glucose into d-gluconic acid thus:



As it turns out, this oxidase, as well as many others, have no known connection with thiamin action, but a somewhat kindred dehydrogenase seems likely to be of importance in this connection, namely, the lactic dehydrogenase which is capable of oxidizing lactic acid to pyruvic acid:

$$CH_3CHOHCOOH - 2\,H \longrightarrow CH_3COCOOH$$

The aerobic oxidases serve to hasten the oxidation by atmospheric oxygen of substances which are slowly subject to oxidation even in the absence of the catalyst. Typical examples are ascorbic acid (vitamin C) and the phenols. Still another substance which has a relationship to both classes of oxidases is cytochrome, which appears to function in the cell as an intermediary between them. Thus oxygen, activated by indophenol, is able to oxidize cytochrome, which can in turn be reduced by various dehydrogenases. The dehydrogenase, after undergoing such oxidation, is thereby restored to its initial condition and is again capable of abstracting hydrogen from the substrate, *e.g.*, lactic acid. Cytochrome may function in this way as the reactivator of succinodehydrogenase also. The latter enzyme converts succinic acid into fumaric, but since this reaction is apparently not related to thiamin function in a direct way, we shall disregard it.

The various enzymes are regarded as consisting of two parts, the one a protein, the other a simpler substance called a coenzyme. In many instances these two component parts of an enzyme can be separated from one another. When separated, neither possesses enzyme activity, but it can often be restored simply by mixing the two. In a number of instances the coenzyme has been isolated and identified and in a few cases has been reproduced synthetically. Of course, we know much less about the protein portions of the enzymes than about their simpler companion substances. Indeed, some may still doubt whether the other portion of an enzyme is truly a protein, but the idea is widely accepted as a general characteristic of enzymes. Whatever its nature, it has very specific properties, such that the "protein" of one enzyme cannot function in another enzyme system. The coenzymes are in general also highly specific, although instances are known in which a single coenzyme, *e.g.*, cozymase, functions in several enzyme systems, but not in a large number of them. We consider the term coenzyme a little unfortunate in that it may be thought to imply an external "activator" of the enzyme while actually it is to be regarded as an integral part of it. Indeed, one is justified in thinking of the coenzyme as the functioning portion and the protein as the modifier rather than vice versa. For more complete information on enzyme systems the reader is referred to a special work on the subject (1).

Deficiency of Oxidases in Tissues

A valuable method for the study of oxidase or dehydrogenase systems has been by the use of methylene blue, which is reduced to the colorless leuco base by those metabolites which play the role of hydrogen donators. Ever since Dutcher (2) suggested that vitamin B_1 might well function as an oxidative catalyst, this idea has been explored by many investigators, several of whom used the methylene blue technique. Although there were many bits of evidence in favor of the theory that there is impairment of oxidation in vitamin B_1 deficiency, the earlier experiments proved inconclusive, partly on account of complications due to the multiple nature of the B complex and still more because of inanition which commonly accompanies experimental polyneuritis. Thus a lowered basal metabolism and diminished CO_2 output in B_1 deficiency could equally well be accounted for as a result of inanition or of impaired oxidative function. Westenbrink (3) has reviewed critically fifteen studies of the subject which made use both of manometric methods and of colorimetric techniques of the methylene blue type and concluded that no significant difference exists as to the respiratory power of polyneuritic tissues as compared with normal ones. Drummond and Marrian (4) accounted for the low body temperature in experimental polyneuritis as due to inanition. When the temperature of the animal was raised by immersion in a warm bath, the metabolic rate and the oxygen consumption rose likewise.

Catatorulin Action of Vitamin B_1

Although these and similar experiments discouraged the protagonists of the theory, the idea of an oxidative catalyst function for vitamin B_1 has not been abandoned. New impetus has been given to it in recent years by the evidence that vitamin C is capable of undergoing oxidation and reduction reversibly. The discovery that riboflavin, vitamin B_2, in the form of its phosphoric ester, constitutes the prosthetic group of an oxidizing enzyme (see Chapter X) also gave it a stimulus. But above all, the theory has held its position on the basis of the long series of experiments by Peters and his associates, who have consistently found a reduced oxygen uptake in polyneuritic tissue, especially brain tissue, which, according to their later results, can be restored toward normal by addition of thiamin *in vitro*. This behavior of thiamin is often referred to as the catatorulin effect.

On the other hand, the still more recent isolation from yeast by Lohmann and Schuster of a pyrophosphoric ester of thiamin which appears to act as a part of a decarboxylating enzyn system introduces a new complication (see Chapter XIV). Specifi-

cally Lohmann and Schuster attribute to it the power to promote a reaction which involves no oxidation:

$$\underset{\text{Pyruvic Acid}}{CH_3COCOOH} \longrightarrow \underset{\text{Acetaldehyde}}{CH_3CHO} + CO_2$$

The essential question at the moment seems to be whether thiamin has to do with oxidation or with decarboxylation or both, and in any case, how the two processes are related to one another as they seem to be in many of the experiments so far performed.

In considering this topic we must first review Peters' work on respiration of brain tissue in some detail, correlating it as fully as possible with the contributions of other workers along parallel lines. Peters has contributed, in recent years, three reviews of the status of the problem (5–7). Each of these reflects successively some alteration of previous viewpoint arising out of new developments of intervening months. The latest (*Chemisch Weekblad*) alone takes into account the discovery of cocarboxylase by Lohmann and Schuster, which was not announced till early in 1937. We shall, therefore, direct our attention to this latest review.

The prominence of the nervous symptoms in polyneuritis and particularly the prompt response to the administration of thiamin raise an insistent question as to the cause of the convulsions and the site of the prompt curative action of thiamin. Having in mind the previous semi-conjectural association of thiamin with carbohydrate metabolism, Kinnersley and Peters (8) were interested in the presence of supernormal amounts of lactic acid in the brain in correlation to a degree with the severity of symptoms. The evidence adduced by Holmes and Holmes (9, 10) is that lactate is oxidized by brain tissue, but is not resynthesized to sugar in the brain. This simplification gave ground for hope that the brain system would prove peculiarly amenable to chemical study of carbohydrate metabolism. To avoid the serious complication of experimental results due to the rapid postmortem formation of lactic acid in the tissue, a technique was devised to apply liquid air to the tissue at the moment of death or to kill the pigeons with iodoacetate, which stops the formation of lactic acid. If the respiration of the tissue is to be studied subsequently, iodoacetate must, of course, be avoided in some cases on account of its inhibiting effect in many enzyme systems.

With such brain preparations in Ringer phosphate solution at *p*H 7.3 in the presence of added glucose, lactate, or, as was subsequently discovered, pyruvate, it was possible to observe a lessened oxygen uptake in polyneuritic brain tissue as compared with normal. The abnormality was most marked in the optic lobes, somewhat less so in the cerebrum, and still less in the rest of the brain. In the absence of added substrates the difference

could not be observed. More striking still was the observation that the addition of thiamin in the form of concentrates or as the pure substance to the medium would increase the oxygen uptake of the polyneuritic tissue *in vitro* (11, 12) (Fig. 18). This effect of thiamin is not observed in brain tissue of normal birds or of starved birds and is evidently specific for polyneuritis. Tissues from scorbutic animals or B_2 deficient animals do not show it. When polyneuritic birds are cured with thiamin, the effect

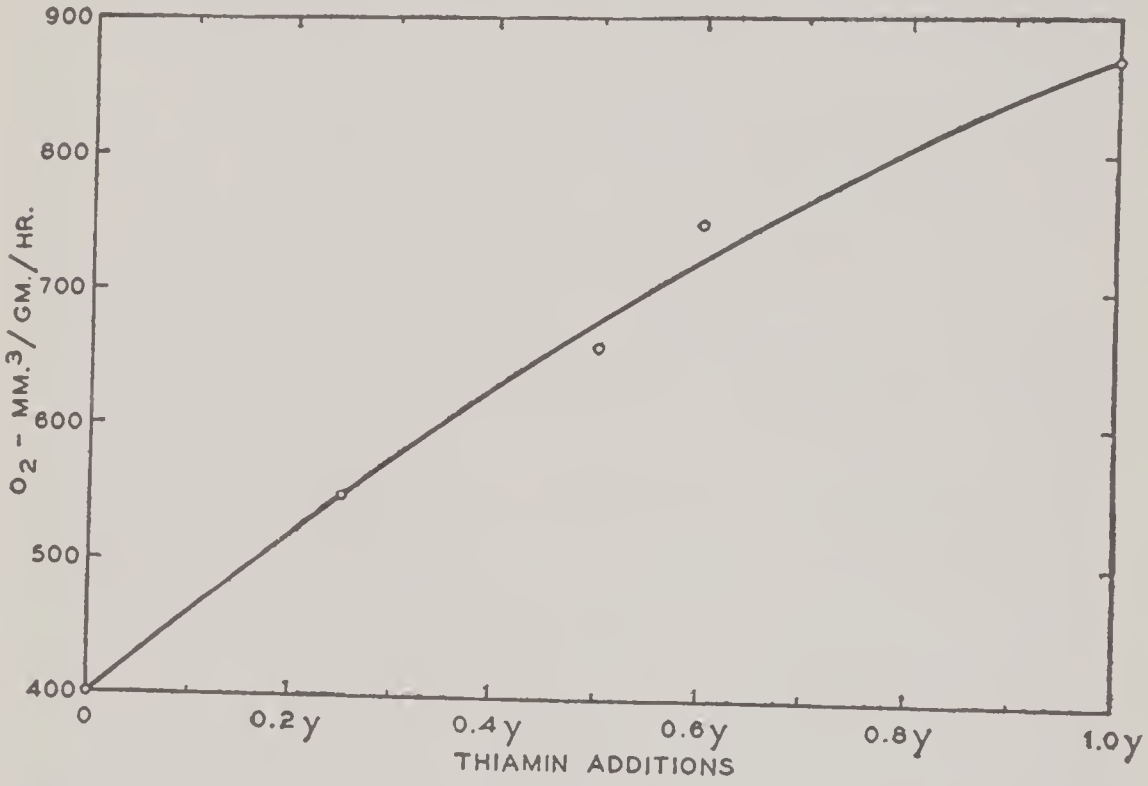


Figure 18. Increase in oxygen uptake *in vitro* of avitaminous pigeon brain tissue with increasing amounts of thiamin chloride. Pyruvate as substrate.

of added thiamin on the respiration of their tissues *in vitro* is diminished (13). One γ of crystalline thiamin can cause 100 milligrams of tissue to take up 0.14 c.c. more oxygen than the control in two hours. This indicates a genuine catalytic effect, since thousands of times more oxygen is taken up than would be consumed in the oxidation of the added thiamin in the ratio of 1 atom of oxygen per mole.

These results appear to support the oxidase coenzyme theory of thiamin action, but it still remains to associate the action with a particular enzyme system. Peters *et al.* repeated earlier work of Drummond and Marrian (4) and failed to detect a diminished oxidation of polyneuritic tissue in a succinate medium. Thiamin evidently does not have to do with oxidation of succinate. Nor does it apparently have to do directly with the oxidation of lactate for, although oxygen uptake on a lactate medium is enhanced by added thiamin, there is no increase in the rate of disappearance of lactate (14). This appears to be an extremely important

observation. Brain systems, like muscle systems, are capable of converting lactate to pyruvate. Moreover, avitaminous brain tissue respiring in lactate medium for two hours produces measurable amounts of pyruvate, and the amount which accumulates is reduced by the presence of added thiamin—also a point of crucial importance. Peters believes, largely on this evidence, that the dehydrogenase which normally converts lactate to pyruvate is unimpaired in polyneuritic brain tissue in spite of the fact that lactic acid accumulates in abnormal amount in such tissue *in vivo*. He accounts for this very reasonably by calling attention to the finding of Green and Brosteaux (15) that pyruvic acid in a concentration of 0.04 *M* inhibits completely the action of lactate dehydrogenase. Accordingly lactate accumulates because pyruvate has done so. Normal brain respires more rapidly in pyruvate than in lactate; polyneuritic brain shows the reverse behavior. The effect of added vitamin on polyneuritic tissue in pyruvate medium is poisoned by fluoride, iodoacetate, or dichlorethylsulfone, but the significance of these facts is not clear.

The catatorulin effect of thiamin is also observed in the tissues of the polyneuritic rat (16) and of the chicken (17). Pyruvate is also found in the blood of avitaminous rats and pigeons (18). Accordingly the phenomenon must be regarded as a general one and characteristic of polyneuritis though perhaps not uniquely so. Peters, however, cautions against the view that the presence of pyruvic acid causes the symptoms. Attempts to produce the symptoms by injection of pyruvate have failed. Also De Jong (19) could not correlate the increase in bisulfite-binding substances in the blood with the severity of symptoms. The failure of normal carbohydrate metabolism in the brain may well be responsible for the symptoms. However, there is still a considerable weight of opinion in favor of the view that incompletely oxidized products of metabolism have toxic effects which are more or less directly responsible for the symptoms. Westenbrink (20) favors such a view and Elvehjem appears to do so without explicitly committing himself.

There seems to be a reasonable ground for doubting whether the attempts to reproduce the symptoms of polyneuritis by injection of pyruvic acid (or methyl glyoxal) have been exhaustive. In view of the large amounts of lactic acid found to be present in the body (21), it might be of interest to try doses of pyruvate as high as 1 to 5 grams per kilo and to utilize experimental animals previously depleted of the greater part of the body's store of thiamin. Nothing could be more illuminating than to be able to bring on typical symptoms by the use of a known intermediate of carbohydrate metabolism. In this connection, attention should be called to the fact that symptoms may often be brought on in

rats, or more particularly in pigeons, which are on the verge of polyneuritis, by frightening the animal into strenuous physical effort.

We may regard the above studies as establishing almost beyond question that there exists a defect in the pyruvic (and incidentally lactic) acid disposal systems of the brain in polyneuritis. Independent evidence exists of the lactic acid disturbance, but it is not flawless. Findlay (22) noted lower glyoxalase content of the liver in polyneuritis and, arguing on the basis of Neuberg's theory that glyoxal (pyruvic aldehyde) is the predominant precursor of lactic acid in muscle, connected the lowered glyoxalase with a lowered carbohydrate metabolism. More direct evidence was supplied by Kinnersley and Peters (23), who not only detected excess lactic acid in the brain but found that the distribution of the excess in various parts of the brain corresponded to the subsequently discovered localization of diminished respiration. Lecoq (24) introduced 5 per cent lactic acid in a B_1 deficient diet and reported earlier development of polyneuritis in pigeons and an enhanced need for yeast to prevent the development of symptoms. Quite contrary results have been reported by Schrader (25) in rats. A poorer utilization of d-glucose was noted in the deficient animals but no definite indication of a breakdown in the lactic acid metabolism. The use of 0.3 gm. to 0.5 gm. daily of lactic acid in the diet did not hasten the onset of the symptoms. Guha (26) found no acceleration of the development of polyneuritis in pigeons by administration of lactate. Thompson and Johnson (27) found large amounts of bisulfite-binding substances in the blood of polyneuritic pigeons and rats, which they considered probably largely pyruvic acid. (See, however, Chapter XXVII regarding the occurrence of methylglyoxal in B_1 deficiency.) Johnson (28) isolated pyruvic acid as a hydrazone from the blood of B_1 deficient pigeons but could not find it with certainty in that of normal birds. A lowered capacity for removal of lactic acid generated by muscular work has been noted in polyneuritic pigeons and in human beriberi (29, 21). Platt and Lu (30) find an increase of pyruvate in the blood stream of human beriberics, but both the results of De Jong, cited earlier, as well as those of Wilkins, Taylor, and Weiss, who also note its presence (31), throw great doubt upon the specificity of its occurrence as an index of B_1 deficiency. We may safely assume that the difficulties of avoiding significant changes in the tissues after death as well as those of analyzing correctly the complex body fluids and tissues have contributed to the conflict of evidence.

Sherman and Elvehjem found no rise in the bisulfite-binding substances in the blood of B_1 deficient chicks, but various tissues and the excreta showed an increase. Injections of pyruvate re-

vealed a more rapid rate of its removal from the blood of normal as compared with avitaminous chicks (32). They also studied anaerobic glycolysis in brain, heart, kidney, liver, and muscle, and established that the formation of lactic from pyruvic acid is normal in avitaminous chick tissue and that there is no anaerobic accumulation of pyruvic acid. Added B_1 had no influence on the results. Its action appears to be limited to the oxidative metabolism of these intermediates (33). (Compare this with Krebs and Lipmann's results discussed below.) This suggests that the pyruvate formed in avitaminosis is produced by an oxidative process, most probably by lactic dehydrogenase, and that the conversion of pyruvate to lactate is not the principal route for disposal of pyruvate. As far as B_1 is concerned, we seem to be dealing with the forward reaction, not the reverse, in the following series:

$$CH_3CHOHCOOH \rightleftharpoons CH_3COCOOH \rightleftharpoons X$$

This is in accord with Peters' view that thiamin has to do with the disposal of pyruvate but does not tell us whether this disposal is an oxidative process. Sherman and Elvehjem also found (17, 34) that while lactate oxidation is not affected by added thiamin in the case of the brain of avitaminous chicks, it is increased in the case of the heart. Further additions of pyruvate inhibited the lactic acid dehydrogenase in avitaminous heart and kidney tissues. Similar respiration studies on a pyruvate substrate showed increased oxygen uptake nearly to normal, by addition of thiamin to cerebrum and kidney tissues from B_1 deficient chicks. This was accompanied by an increased removal of pyruvate. Thus, in a general way, Peters' results, which have been confirmed elsewhere, are shown to be more or less applicable to other polyneuritic tissues than the brain and to other species than the pigeon.

Peters has been strongly disposed, presumably because of the extent of his experiments on the effect of thiamin on oxygen uptake, to believe that pyruvic acid is disposed of by an oxidative process which thiamin somehow catalyzes. However, he calls attention to the fact that thiamin has not been found to decolorize methylene blue (35). Lipmann has dehydrogenated thiamin (36) and Barger and others have oxidized it to thiochrome, but neither of these reactions appears to be reversible. Krebs (37) has suggested that thiamin acts as a promoter of a dismutation:

$$2\,CH_3COCOOH + H_2O \longrightarrow CH_3COOH + CO_2 + CH_3CHOHCOOH$$

in which one molecule of pyruvic acid is reduced to lactic while the other is oxidized and decarboxylated. However, in a later paper having to do with the behavior of staphylococcus and gonococcus in pyruvate media (38), Krebs reports that many substances, including Warburg's yellow enzyme and coenzyme

as well as fumaric and ketoglutaric acids, activate this dismutation. Crude preparations of thiamin, perhaps containing the pyrophosphoric ester, promoted it, but pure thiamin did not. Moreover, Peters (39) was not able to parallel in brain tissue Krebs' earlier experimental results with cocci, and in a recent paper (40) Krebs is reported to have abandoned the idea himself. However, Hills resolutely adheres to the view that Staphylococcus causes such a dismutation and that it is promoted by the presence of thiamin (41). He attributes Krebs' failure definitely to detect such an effect of thiamin to the presence of thiamin in the basal medium (broth agar). By use of modified medium, Hills is able to demonstrate the effect (42).

McGowan (43) found that the pyruvate which disappears in respiration experiments with normal brain in pyruvate phosphate medium is in excess of the equivalent of the oxygen which would be consumed by the reaction:

$$CH_3COCOOH + 5O \longrightarrow 3\,CO_2 + 2\,H_2O$$

From his experiments one would conclude that about one fifth or more of the pyruvic acid escapes complete oxidation. The respiratory quotient for washed normal brain was found to be about 1.30, whereas the theoretical for pyruvic acid is 1.20. This indicates that the pyruvic acid is incompletely oxidized in normal brain. When the experiments on oxygen pyruvate ratio were repeated on avitaminous brain in the presence of added thiamin, the oxygen consumed was even less adequate to account for the pyruvate which disappeared.

The paper by McGowan and Peters which is cited above discusses the question whether pyruvate (*a*) is directly oxidized, (*b*) combines with itself or with some other substance to form an intermediate which then undergoes oxidation, or (*c*) combines with thiamin to form an intermediate which is then oxidized. They did not find that oxygen uptake was increased by adding keto acids (α ketoglutaric, α ketoadipic, acetoacetic, or succinnic) to pyruvate substrate and conclude that the oxidase system in which thiamin plays a part is not a general α keto oxidase but is specific for pyruvate. This would seem to differentiate this enzyme system from the carboxylase of Neuberg, which acts on keto acids generally.

The pyruvate oxidase coenzyme theory of thiamin action does not appear to be making much headway, but the incontrovertible fact remains that a large increase in oxygen uptake accompanies the removal of pyruvate by brain tissue.*

* A further suggestion appears as we go to press. It is based upon the finding of large amounts of citric and α ketoglutaric acids in the urine of polyneuritic rats. These are regarded as intermediates in the breakdown of pyruvic acid. Krebs. Chem. and Ind. *57*, 212, 1938.

than as a coenzyme for carboxylase, which, as Neuberg described it, converts pyruvic acid to CO_2 and acetaldehyde. He believes that the colloidal carrier of the carboxylase is different from that of the dehydrogenase. The former is present in yeast, the latter in the bacteria with which Lipmann experimented. With these bacteria he found that the anaerobic decomposition of pyruvic acid was only about one-fifth as great as the aerobic as judged by oxygen uptake, CO_2 evolution, and pyruvate disappearance. With well-washed bacteria the anaerobic disappearance of pyruvate could be brought nearly to zero. From these facts, including the existence of a respiratory quotient of about 2, Lipmann concludes that a pure dismutation or a pure decarboxylation is out of the question, and that there is simultaneous loosening of hydrogen atoms as decarboxylation occurs in a single reaction:

$$CH_3COCOOH \longrightarrow CH_3COOH + CO_2$$

Free thiamin does not function in this system, only its pyrophosphate. Because of this fact, we do not like to refer to thiamin pyrophosphate as cocarboxylase. This name emphasizes only one aspect of its physiological activity and should be reserved for that aspect.

Possible Synthesis of Fat from Pyruvic Acid

Still another line of suggestion as to the role of thiamin is now developing. McHenry (57) reported a complementary action of choline and thiamin in increasing the weight of young rats. However, an increase in the liver fat of the animals kept on a low-choline diet was induced by administration of thiamin. Choline has been shown to decrease the liver fat (58); the two substances thus have opposite effects on fat deposition. McHenry's diets covered a wide range of fat content, from 0 to 60 per cent. The liver fat on a given intake of thiamin was found to rise regularly with the percentage of dietary fat, even when the food intake was controlled at an even level on the paired feeding method (59). The effect of thiamin on liver fat was also marked on fat-free diets. Further, the body fat of the animals paralleled in a general way the liver fat and produced a quite significant effect on total body weight (an effect which became very marked if the animals were allowed to eat *ad libitum*). In a preliminary experiment the removal of the hypophysis eliminated the effect of B_1 on the accumulation of liver fat.

Similar results as to body fat had been briefly reported a few weeks previously by Whipple and Church (60). In paired feeding experiments using fat-free diets, rats developed 9.4 per cent body fat with B_1 addendum and only 3.3 per cent fat without B_1. The authors suggested that B_1 plays a role in converting

carbohydrate to fat. These authors have since pursued their studies (61) by observing the respiratory quotients of normal and deficient rats after injection of glucose. The former averaged 1.15, the latter 0.85. When polyneuritis developed, the respiratory quotients averaged 0.79 but rose on the injection of 0.1 mg. of thiamin to 1.48. As the characteristic respiratory quotient for combustion of fat is 0.7, protein 0.8, and carbohydrate 1.0, a higher quotient than 1.0 is evidence that another reaction is in progress. This might be the combustion of substances already partly oxidized (*e.g.*, pyruvic acid, R.Q. = 1.2) or the utilization of oxygen of carbohydrate for combustion of some of its own carbon atoms, the remaining atoms being reduced to alkyl chains. The latter condition would be fulfilled by the conversion of carbohydrate to fat. The above, therefore, is essential for synthesis of fat in the body.

There are in the literature some conflicting data on the respiratory quotient in thiamin deficiency. In general, it has been found to be lowered. Abderhalden and coworkers (1920–1922) and Caridroit (1922) found values of 0.75 to 0.78; Ramoino (1916) 0.44. Van Berkhout (1926) found a lowered CO_2 output, which, however, Drummond and Marrian attributed to the inactivity of the sick birds (4). G. A. Schrader (25) reports a respiratory quotient of 1.26 in B_1 deficient rats as well as in normal ones on a high carbohydrate diet.

In a more recent communication McHenry (62) says, "There is no doubt that the vitamin permits the oxidation of pyruvic acid by brain tissue *in vitro,* and the explanation may be completely satisfactory for that tissue." He then goes on to propose the hypothesis that fat is synthesized in the presence of thiamin, utilizing pyruvic acid as the intermediate. In this connection he refers to the work of Krebs and Johnson (63), showing that hydroxybutyric acid can be formed from pyruvic acid by tissues. This hypothesis appears to be very attractive, as it seems to hold the possibility of reconciling many divergent observations. Notably, it offers a concrete idea of the mechanism of the B_1 sparing action of fat which is discussed at some length in Chapter XXI.

The substance of those aspects of the above-mentioned paper by Krebs and Johnson with which we are especially concerned is as follows: The main anaerobic action in brain is:

(1) $$2 \text{ pyruvic acid} + H_2O \longrightarrow \text{acetic acid} + CO_2 + \text{lactic acid}$$

Other reactions by which α ketonic acids, including pyruvic acid, are oxidized and decarboxylated in the tissues are:

(2) $$RCOCOOH + R'COCOOH + H_2O \longrightarrow RCOOH + CO_2 + R' \cdot CHOH \cdot COOH$$

(3) $$\underset{\beta \text{ Ketonic Acid}}{RCOCOOH + R'COCH_2COOH} + H_2O \longrightarrow RCOOH + CO_2 + R'CHOHCH_2COOH$$

Acetic acid from (1) is converted to acetoacetic acid which, reacting by dismutation with pyruvic acid, produced β hydroxybutyric acid, CO_2, and acetic acid. A summation of the several successive reactions involving pyruvic acid is

(4) 3 pyruvic + H_2O ⟶ β hydroxybutyric acid + lactic acid + 2 CO_2

This indicates that pyruvic acid and other ketone bodies are intermediates not only in fat but also in carbohydrate metabolism.

A weakness of the Krebs-Johnson work for our purposes is that pyruvic acid was determined by the carboxylase method, which is scarcely specific enough to convince the skeptical. A strong feature of the paper is that it involved many different tissues from many different species and yielded consistent results. The tissues were, however, normal rather than polyneuritic ones, as the primary purpose of the study was not related specifically to thiamin function.

These authors refer to preliminary experiments intended to determine whether thiamin is capable of promoting the anaerobic dismutation of pyruvic acid. Pure thiamin did not do so, but crude material from natural sources, perhaps containing the pyrophosphoric ester, did. (Compare this with the previously cited result of Lipmann in which free thiamin failed to promote oxygen uptake while the cocarboxylase did so.)

It may seem disappointing that the chemical structure of thiamin has not proven suggestive as to its function. None of the functions proposed for it has an evident relationship to any feature of the structure of the substance. However, this seems to be equally true of all the other vitamins which have been identified structurally. One is tempted in this instance to speculate upon the possible significance of the resemblances of thiamin and choline, since both are quaternary bases and substituted ethyl alcohols.

We cannot, of course, accept without further evidence the idea of a fat synthesis function of thiamin, much as we are attracted by its plausibility. An adequate theory must take into account its function in the germinating seed, particularly in the growth of roots. This phenomenon is discussed in Chapter XXVIII. The fat synthesis theory has some attractive features in this connection. Seeds are often classified into two groups, of which one stores food as fat, the other as starch. The conversion of starch to fat in the ripening of fatty seeds is well known. During germinating of fatty seeds, fat disappears rapidly and the respiratory quotient is very low, often less than 0.5. The starchy seeds likewise give evidence of metabolizing fat, for the respiratory quotient is prevailing below unity. This suggests the conversion of carbohydrate to fat during germination. Pending

further investigation, we can only say that the fat synthesis theory of thiamin function is not inconsistent with what we know of the role of the substance in plants.

One of the latest studies of brain respiration is that of Lipmann (64), which is summarized by the same author elsewhere. Lipmann used brain tissue in bicarbonate rather than Ringer phosphate solution and utilized glucose instead of pyruvic acid as the substrate. Under these conditions, avitaminotic brain produced pyruvic acid. Fructose, which is not split glycolytically by brain enzymes, nevertheless yielded pyruvic acid also when subjected to the action of avitaminotic brain. This is taken to indicate the formation of pyruvic acid direct from the carbohydrate. Addition of thiamin to the substrate prevented the accumulation of pyruvic acid and also reduced the aerobic formation of lactic acid. The effect of thiamin addition to avitaminotic brain in presence of oxygen was marked, especially after respiration had gone on for some time. Without thiamin, respiration soon fell off. In anaerobic experiments pyruvic acid underwent dismutation to acetic acid, carbon dioxide, and lactic acid as conceived by Krebs, but thiamin did not influence the course of this anaerobic reaction. Lipmann's experiments included many measurements under varied conditions but did not involve thiamin pyrophosphate, although he discusses the latter briefly. His conclusion is that the products of the oxidative process are acetic acid and carbon dioxide; that pure decarboxylation which is promoted by thiamin pyrophosphate in the presence of yeast is absent in the case of animal tissue.

Reconciliation of the Divergent Findings

All tissue respiration experiments, such as we have discussed at length, necessitate a great deal of interpretation by inference. When a single enzyme system is operating and single reaction is in process, the inference from oxygen consumption, carbon dioxide, disappearance of substrate, etc., may become quite trustworthy, especially if two or more independent sets of data point in the same direction. However, when respiratory quotient, for example, does not quite agree with the disappearance of substrate, the co-occurrence of two or more reactions is to be suspected. Rarely is it possible to isolate intermediate products quantitatively for purposes of confirmation. The reader, therefore, should not lose faith in the results because of conflict as to details. Much further exploration may be required before the distinction between primary and secondary phenomena becomes clear.

It also must be evident to the reader that while there is a great deal to support the view that the pathways by which glu-

cose is utilized in various organisms are closely interrelated and are in part identical, there is often great difficulty in assuring oneself in any given case that he is dealing with a general phenomenon rather than with an exceptional peculiarity of the tissue or system he has in hand. Thus one cannot with safety transfer the findings as to the metabolism of bacteria, for example, to the case of pigeon brain and vice versa. If one could do this with assurance, Lipmann's theory might be fitted to Peters' results satisfactorily as far as we can now tell. It is true that the oxygen consumption per mole of pyruvate observed by McGowan and Peters is much higher than that called for by Lipmann's reaction, but the excess might be accounted for by other oxidations, *e.g.*, lactic acid or other dehydrogenation. Enzyme systems are often closely linked together (65).

Peters' idea of thiamin as a pyruvate oxidase cannot yet be dismissed. In a late paper (66) he reports that free thiamin has under his conditions a tenfold greater effect on oxygen uptake of polyneuritic brain tissue than the pure pyrophosphate obtained from Lohmann. It has occurred to the present authors that it would be of interest to determine whether thiamin stimulates oxygen uptake of polyneuritic tissues on any substrate of fewer carbon atoms than pyruvic acid. Thus if thiamin promotes the oxidation of pyruvic acid to carbon dioxide and water, it might affect the oxidation of acetaldehyde or ethyl alcohol. The numerous studies of the etiology of alcoholic polyneuritis clearly imply that thiamin is needed for the metabolism of ethyl alcohol, just as it is needed for the metabolism of carbohydrates. Otherwise the substitution of alcohol for carbohydrate or proteins or both would not occasion polyneuritis. Further, a greater persistence of alcohol in the blood stream of beriberic pigeons than in that of normals has been noted (67). Oxidation of acetic acid is apparently not one of the functions of thiamin, as addition of this acid to the medium in the catatorulin test does not enhance the oxygen uptake according to McGowan and Peters.

A possible defect in Peters' method might seem to be the use of unwashed tissue which could accordingly furnish substrate apart from the pyruvic acid of the medium. This, Peters feels, he has excluded since without added substrate there is no difference in the action of thiamin on normal brain tissue as compared with polyneuritic. Another possibility which may require further consideration is a possible linking of riboflavin with thiamin in the pyruvate oxidase system. Pigeons rendered polyneuritic on polished rice are certainly deficient in both vitamins, and there is reason to think beriberi may involve both deficiencies (see Chapter II).

We are compelled by a consideration of all the enzyme

studies which have appeared to suppose that thiamin has a multiple function as a coenzyme in nature. The best-established ones are the two known reactions of its pyrophosphate. This, in association with the protein of yeast, can bring about a pure decarboxylation of pyruvic acid or, in association with other proteins, it can act to promote a simultaneous dehydrogenation and decarboxylation of pyruvic acid to form acetic acid and carbon dioxide. Less clearly established are the purely oxidative function, which may be a property of free thiamin jointly with its pyrophosphate, and a conversion of carbohydrate to fat, which may be associated with either form as far as we know. One of these reactions may predominate in one tissue, another in another tissue. Some of the reactions may be limited to certain specialized tissues of the animal body. These conclusions are tentative and may require an early revision.

It is not unreasonable to suppose that thiamin in the form of its pyrophosphoric ester acts as a coenzyme for several enzymic systems, though probably not for a large number. Thus the same coenzyme, cozymase, is believed to function not only in zymase but in several animal dehydrogenases acting upon hexosemonophosphate, succinic and formic acid and alcohol, and also, perhaps, in lactic acid formation. Such a multiple function for thiamin pyrophosphate would be certain to produce a considerable temporary confusion.

Summary

1. Thiamin, perhaps in the free state, more certainly in the form of its pyrophosphate, serves as a means of disposal of pyruvic acid and, indirectly, of lactic acid in the normal carbohydrate metabolism.

2. The phenomenon is enzymic and is accompanied by increase of carbon dioxide evolution and often by increase of oxygen uptake. The relative predominance of the two varies from one tissue to another.

3. A coenzyme function of thiamin pyrophosphate is thus established.

4. A secondary consequence of pyruvic acid removal in polyneuritic tissues is the dissipation of lactic acid, the dehydrogenation of which is inhibited by excess pyruvate.

5. Its functions appear to include that of a coenzyme for pyruvate oxidase (Peters), a coenzyme for a pure carboxylase (Lohmann), a coenzyme for a dehydrogenase-carboxylase (Lipmann), and, less probably, according to present evidence, a coenzyme for an anaerobic dismutase (Krebs).

6. A role for thiamin or its pyrophosphate as a coenzyme in the synthesis of fat from carbohydrate via pyruvic acid is an

attractive possibility though apparently at variance with the weight of present evidence gained from tissue respiration experiments. This is in favor of an aerobic function in animal tissues.

BIBLIOGRAPHY

1. Tauber, H: Enzyme Chemistry. John Wiley and Sons, New York, 1937.
2. Dutcher, R. A.: Vitamin Studies. I. Observations on the Catalase Activity of Tissues in Avian Polyneuritis. Jour. Biol. Chem. *36*, 63, 1918.
3. Westenbrink, H. G. K.: Ist die Gewebsatmung bei Beriberi erniedrigt? Arch. Neerl. d. Physiol. *17*, 239 and 549, 1932.
4. Drummond, J. C., and Marrian, G. F.: The Physiological Role of Vitamin B. I. The Relation of Vitamin B to Tissue Oxidation. Biochem. J. *20*, 1229, 1926.
5. Peters, R. A.: Biochemical Lesion in Vitamin B_1 Deficiency. Application of Modern Biochemical Analysis and Its Diagnosis. Lancet *230*, 1161, 1936.
6. Peters, R. A.: The Contribution of Vitamin B_1 to the Metabolism of the Brain. Chemisch Weekblad *34*, 442, 1937.
7. Peters, R. A.: Vitamin B_1 (Oryzanin, Torulin, Aneurin). Its Chemistry and Mode of Action. Current Science *5*, 207, 1936.
8. Kinnersley, H. W., and Peters, R. A.: Observations upon Carbohydrate Metabolism in Birds. I. The Relation between the Lactic Acid Content of the Brain and the Symptoms of Opisthotonus in Rice Fed Pigeons. Biochem. J. *23*, 1126, 1929.
9. Holmes, B. E., and Holmes, E. G.: Contributions to the Study of Brain Metabolism. Carbohydrate Metabolism. Biochem. Jour. *19*, 492, 1925.
10. Holmes, B. E., and Holmes, E. G.: Carbohydrate Metabolism of the Brain Tissue of Depancreatised Cats. Biochem. Jour. *21*, 412, 1927.
11. Gavrilescu, N., and Peters, R. A.: On the Function of Torulin. An *in Vitro* Effect of Antineuritic Vitamin Concentrates. Biochem. Jour. *25*, 2150, 1931.
12. Gavrilescu, N., Meiklejohn, A. P., Passmore, R., and Peters, R. A.: Carbohydrate Metabolism in Birds. The Site of the Biochemical Lesion in Avian Polyneuritis. Proc. Roy. Soc. Lond. *B110*, 431, 1932.
13. Meiklejohn, A. P., Passmore, R., and Peters, R. A.: The Independence of Vitamin B_1 Deficiency and Inanition. Proc. Roy. Soc. Lond. *B111*, 391, 1932.

14. Meiklejohn, A. P.: Avian Polyneuritis. Further Studies on the Action of Vitamin B_1 Concentrates in Vitro. Biochem. J. *27,* 1310, 1933.
15. Green, D. E., and Brosteaux, J.: The Lactic Dehydrogenase of Animal Tissues. Biochem. J. *30,* 1489, 1936.
16. O'Brien, J. R., and Peters, R. A.: Vitamin B_1 Deficiency in the Rat's Brain. Jour. Physiol. *85,* 454, 1935.
17. Sherman, W. C., and Elvehjem, C. A.: In Vitro Action of Crystalline Vitamin B_1 on Pyruvic Acid Metabolism in Tissues from Polyneuritic Chicks. Amer. Jour. Physiol. *117,* 142, 1936.
18. Thompson, R. H. S., and Johnson, R. E.: Blood Pyruvate in Vitamin B_1 Deficiency. Biochem. J. *29,* 694, 1935.
19. De Jong, S.: Bisulphite-binding Capacity of the Blood of Pigeons with B_1 Deficiency. Arch. Neerl. Physiol. *21,* 465, 1936.
20. Westenbrink, H. G. K.: Über die Physiologische Wirkung des Vitamins B_1. Arch. Neerl. Physiol. *20,* 481, 1935.
21. Fisher, R. B.: Carbohydrate Metabolism in Birds. Effects of Rest and Exercise upon Lactic Acid Content of Organs of Normal and Rice-Fed Pigeons. Biochem. J. *25,* 1410, 1931.
22. Findlay, G. M.: Glyoxalase in Avian Beriberi. Biochem. J. *15,* 104, 1921.
23. Kinnersley, H. W., and Peters, R. A.: A Localized Lactic Acidosis in the Brain of Pigeons Suffering from B_1 Deficiency. Jour. Physiol. *69,* XI, XII, 1930.
Carbohydrate Metabolism in Birds. II. Brain Localization of Lactic Acidosis in Avitaminosis B and Its Relation to the Origin of Symptoms. Biochem. J. *24,* 711, 1930.
24. Lecoq, R.: L'Imprégnation Lactique des Tissus est-elle la Veritable Cause de la Polynévrite Aviaire? Compt. Rend. Soc. Biol. *120,* 958, 1935.
25. Schrader, G. A.: The Ability of the Vitamin B-deficient Rat to Utilize d-Lactic Acid. Ala. Agr. Exp. Sta. Ann. Rept. *45,* 22, 1934.
26. Guha, B. C.: The Physiological Function of Vitamin B_1. Biochem. J. *25,* 1367, 1931.
27. Thompson, R. H. S., and Johnson, R. E.: Blood Pyruvate in Vitamin B_1 Deficiency. Biochem. J. *29,* 694, 1935.
28. Johnson, R. E.: Isolation of Pyruvic Acid from the Blood of Vitamin B_1 Deficient Pigeons. Biochem. J. *30,* 31, 1936.
29. Inawashiro, R., and Hayasaka, E.: Studies on Effect of Muscular Exercise in Beriberi; Influences of Muscular Exercise upon Gas and Carbohydrate Metabolism. Tohoku Jour. Exp. Med. *12,* 1, 1928.
30. Platt, B. S., and Lu, G. D.: Chemical and Clinical Findings in Beriberi with Special Reference to Vitamin B_1 Deficiency. Quart. Jour. Med. *5,* 355, 1936.
31. Wilkins, R. W., Taylor, F. H. L., and Weiss, S.: Bisulphite Binding Substances in the Blood in Health and in Disease, Par-

ticularly Vitamin B_1 Deficiency. Proc. Soc. Exp. Biol. Med. *35*, 584, 1937.

32. Sherman, W. C., and Elvehjem, C. A.: Effect of Polyneuritis in Chicks upon *in Vivo* Rate of Removal of Pyruvate Injected Intravenously. Jour. Nutrition *12*, 321, 1936.
33. Sherman, W. C., and Elvehjem, C. A.: Study of Anaerobic Glycolysis in Tissues from Polyneuritic Chicks; Negative Action of Vitamin B_1. Amer. Jour. Physiol. *117*, 151, 1936.
34. Sherman, W. C., and Elvehjem, C. A.: *In Vitro* Studies on Lactic Acid Metabolism in Tissues from Polyneuritic Chicks. Biochem. J. *30*, 785, 1936.
35. Rydin, H.: Thesis, Upsala, 1925.
36. Lipmann, F.: Hydrogenation of Vitamin B_1. Nature *138*, 1097, 1936.
37. Krebs, H. A.: Intermediate Metabolism of Carbohydrates. Nature, *138*, 288, 1936.
38. Krebs, H. A.: Dismutation of Pyruvic Acid in *Gonococcus* and *Staphylococcus*. Biochem. J. *31*, 661, 1937.
39. Peters, R. A.: Carbohydrate Metabolism. Nature, *138*, 552, 1936.
40. McGowan, G. K., and Peters, R. A.: Nature, Specificity and Course of Oxidation Catalysed by Vitamin B_1. Biochem. J. *31*, 1637, 1937.
41. Hills, G. M.: Aneurin and Pyruvate Metabolism in *Staphylococcus aureus*. Chem. and Ind. p. 1055, Nov. 1937.
42. Hills, G. M.: Aneurin (Vitamin B_1) and Pyruvate Metabolism by *Staphylococcus aureus*. Biochem. J. *32*, 383, 1938.
43. McGowan, G. K.: Pyruvate Oxidation in Brain. The Oxygen/Pyruvate Ratio and R.Q. Biochem. J. *31*, 1627, 1937.
44. Lohmann, K.: Beziehungen zwischen Fermenten and Vitaminen. Acta. Brevia. Neerland. *7*, 108, 1937.
45. Lohmann, K., and Schuster, Ph.: Untersuchungen über die Cocarboxylase. Biochem. Zeit, *294*, 188, 1937.
46. Westenbrink, H. G. K., and Polak, J. J.: Some Experiments on the Catatorulin Effect. Rec. trav. chim. Pays Bas *56*, 315, 1937.
47. Simola, P. E.: Über den Co-Zymase- und Co-Carboxylasegehalt des Ratten Organismus bei B-Avitaminose. Biochem. Zeit. *254*, 229, 1932.
48. Kinnersley, H. W., and Peters, R. A.: Antineuritic Yeast Concentrates. IV. The Further Purification of Yeast Vitamin B_1 (Curative). Biochem. J. *22*, 419, 1928.
49. Auhagen, E.: Co-carboxylase, ein neues Co-Enzym der alkoholischen Gärung, Zeit. physiol. Chem. *204*, 149, 1932.
50. Stern, K. G., and Hofer, J. W.: Synthesis of Cocarboxylase, from Vitamin B_1. Science *85*, 483, 1937.
51. Tauber, H.: Enzymic Synthesis of Cocarboxylase. Science *86*, 180, 1937.

52. Tauber, H.: Enzymic Synthesis of Cocarboxylase. Enzymologia *2*, 171, 1937.
53. Tauber, H.: Co-carboxylase (Vitamin B_1 Pyrophosphate) Content of Plants. Proc. Soc. Biol. and Med. *37*, 541, 1937.
54. Lipmann, F.: Dehydrierung des Brenztraubsäure. Enzymologia, *4*, 65, 1937.
55. Lipschitz, M. A., Potter, V. R., and Elvehjem, C. A.: Relation of Vitamin B_1 to Cocarboxylase. Biochem. J. *32*, 474, 1938.
56. Lipmann, F.: Pyruvic Acid Dehydrogenation. Vitamin B_1 and Cocarboxylase. Nature *140*, 25, 1937.
57. McHenry, E. W.: An Effect of Choline upon the Weights of Young Rats. Jour. of Physiol. *85*, 343, 1935. Vitamin B_1 and Fatty Livers. Jour. of Physiol. *86*, 27 P, 1936; *89*, 287, 1937.
58. Best, C. H., and Huntsman, M. E.: Effect of Choline on Liver Fat. Jour. Physiol. *83*, 255, 1935.
59. Sure, B., Kik, M. C., and Smith, M. E.: The Specific Effect of Vitamin B on Growth. Jour. Nutrition *5*, 155, 1932.
60. Whipple, D. V., and Church, C. F.: The Composition of Growth Induced by Vitamin B (B_1). Proc. Amer. Soc. Biol. Chem. *30*, cvii, 1936.
61. Whipple, D. V., and Church, C. F.: Effect of Vitamin B_1 on the Respiratory Quotient. Proc. Amer. Soc. Biol. Chem. *31*, ciii, 1937.
62. McHenry, E. W.: Vitamin B_1 and the Synthesis of Fat from Carbohydrate. Science *86*, 200, 1937.
63. Krebs, H. A., and Johnson, W. A.: Metabolism of Ketonic Acids in Animal Tissues. Biochem. J. *31*, 645, 1937.
64. Lipmann, F.: Metabolism of Pyruvic Acid and the Mechanism of the Vitamin B_1 Action. Skand. Arch. Physiol. *76*, 255, 1937. Biological Oxidations and Reductions. Ann. Review of Biochemistry, *VI*, 19, 1937.
65. Das, N. B.: Identity of Lactic and Malic Dehydrogenases. Studies on the Inhibition of the Succinic and Lactic Malic Dehydrogenases. Biochem. J. *31*, 1116 and 1124, 1937.
66. Peters, R. A.: Pyruvate Oxidase in Brain. IV. Cocarboxylase. Biochem. J. *31*, 2240, 1937.
67. Galamini, A.: Blood-alcohol Curve and Experimental Beriberi. Atti. Accad. Lincei. *23*, 623, 1936.

CHAPTER XXVII

CONCOMITANT CHEMICAL DERANGEMENTS

At first glance one might suppose that a well-defined condition of known chemical origin, such as beriberi, would present a definite picture of limited and specific derangement of chemical function. Anatomically and clinically there appears to be a central and fundamental feature of this character, namely, the widespread neuritis associated with fatty degeneration of the myelin sheath. However, while this is central, it is by no means the sole disturbance viewed clinically or anatomically. The upset is so general that visible derangement is encountered in most of the tissues and in a great many functions (see Chapter II). It has indeed often been suggested that concomitant disturbances, such as those of the digestive and cardiovascular systems, are secondary consequences of the nervous derangement.

This view cannot at present be excluded as impossible, but there is another view which seems preferable. This is, that a breakdown of metabolism is primary and that other disturbances, including the nervous ones, are secondary. The widespread character of the symptoms can, according to this view, be ascribed to the fact that the metabolic process concerned is one that involves all functional cells. Disturbance of it, therefore, directly affects all tissues, though not equally. If the conversion of carbohydrates to fats is the central metabolic disturbance, the predominance of the nervous symptoms might be plausibly connected with the conspicuously fatty nature of brain and nerve tissue.

The question is one which cannot be settled experimentally for the present, both because we lack knowledge of the true course of intermediate carbohydrate and fat metabolism and because we cannot yet exercise a sufficient control over external factors. In experimental beriberi in animals one can perhaps approximate a pure thiamin deficiency, but it is doubtful if this has ever been fully achieved. Even if a single deficiency is granted, the adverse effect of this deficiency upon the appetite presently results in a high degree of inanition and many of the pathological changes which ensue are such as to make it diffi-

cult to decide to what extent they are due to the deficiency of thiamin *per se* and to what extent to inanition. In human beriberi one is always in doubt as to whether the deficiency which exists involves only one vitamin. Indeed in many cases there is positive evidence of multiple deficiency. This extends to such a degree that numerous cases are on record of mixed beriberi and scurvy, or mixed beriberi and pellagra. Indeed many cases of each of these diseases show some degree of response upon administration of thiamin. Platt and coworkers hold that secondary deficiencies are prevailingly present in beriberi. Perhaps the most outstanding discrepancy between human and experimental beriberi is the relative absence of inanition in the human disease. Quite possibly this is connected with the incompleteness of the thiamin deficiency in the human dietaries involved. In rats it has been found that an inadequate supply of thiamin is for some unknown reason more conducive to polyneuritis than a complete lack of it. Another discrepancy between the human and the experimental disease is the wider prevalence of edema in the former, a fact for which there is no established explanation (see Chapter IV, page 67).

In view of these complications we can do no better in this chapter than to record the principal biochemical abnormalities which have been associated with thiamin deficiency without attempting to give a final critical appraisal of their true significance. Many of these chemical disturbances have already been mentioned in other chapters and will not be repeated here. They include a frequent but inconsistent modification of glycogen storage and a hyperglycemia, together with excessive amounts of lactic and pyruvic acids in the blood.

Loss of appetite is so conspicuous in experimental beriberi that many students, notably Cowgill, have viewed it as an almost specific symptom of thiamin deficiency. This seems to be a somewhat extreme view. A deficiency of any of the vitamins, as well as other causes, may lead to loss of appetite. Graham and Griffith (1) have adduced evidence that the aggregate of thermostable factors are important appetite stimulants for rats. More specifically, the filtrate factor has been shown to have a powerful influence in this direction (2). This may well be connected with the fact that loss of appetite is one of the first symptoms of pellagra. That thiamin deficiency has such an influence in high degree cannot be doubted. It has been particularly emphasized by Sure (3) and even more by Cowgill (4). Harris, Clay, Hargreaves, and Ward have made a very interesting study of the relation of appetite to instinctive choice of diet by B_1 deficient rats, as a result of which they conclude that the choice is made by association of the beneficial effects of the B_1 rich diets with their characteristic odor, taste, etc. (5).

It is, however, the universal experience of laboratory workers that thiamin, administered apart from food either parenterally or orally, leads to a restoration of appetite in all species of B_1 deficient animals. The response is usually so prompt and pronounced that it seems miraculous when first witnessed. The loss of appetite during the deficiency is associated with an incapacity to digest and assimilate food. Thus, forced feeding as an experimental method to avoid inanition is commonly more or less defeated by the accumulation of undigested food in the alimentary tract. Furthermore, the incapacity to metabolize food does not appear to be confined to this tract. The stoppage is even further along, *i.e.*, in the tissues themselves, so that the loss of appetite may be regarded as the back pressure of a long series of halted processes. Peters speaks graphically of the internal hunger of the tissues.

Disturbances of Digestion

Nevertheless, there has been much search for a specific disturbance in the alimentary tract. Thus, loss of appetite has been attributed to gastric or intestinal atony (6). An enlargement of the colon in rats on B_1 deficient diets was reported by Sparks and Collins (7). Gastric dilatation which responded to thiamin treatment was noted by Moore and Plymate (8). Dalldorf and Kellogg (9) have reported a marked association of thiamin deficiency and gastric ulcer, but opinion as to the effect of the deficiency on the alimentary tract has not been unanimous by any means (10, 11). This matter is more fully discussed in Chapter IV.

A reduced resorption of glucose by the intestine in thiamin deficiency, thermostable B deficiency, or B complex deficiency characterizes these conditions (12), but it does not appear to be clear that this is not a consequence of attendant inanition.

Turning to the chemical evidence of disturbance in the alimentary tract, we find the data quite meager. Webster and Armour (13) note a marked drop in secretion of gastric juice in dogs on B deficient diets, but these authors did not definitely associate the drop with B_1 in particular, but only with yeast vitamins in general. Farnum (14) finds reduction of total volume and of free acidity in gastric secretion in experimental beriberi, and Skarzynska-Gutowska (15) was able to induce a flow of gastric juice by injecting extracts containing thiamin. Ohta (16) found a reduced gastric acidity very prevalent in infantile human beriberi. Joffe and Jolliffe (17) analyzed achlorhydria in alcohol addicts who often exhibit thiamin deficiency and concluded that an achlorhydria preventive factor is probably present in the B complex, but that it is neither thiamin nor the filtrate factor. Others (18) concur in the belief that thiamin deficiency does

not produce very significant reduction of the gastric acidity. However, Masuko (19), studying the matter in dogs by means of a Pavlov pancreatic fistula, noted a marked increase in volume of secretion and enzymic concentration on giving vitamin B. The effect was not noted with thiamin alone and was believed to be due to some combination of B factors.

An exploration of the *p*H throughout the intestinal canal of rats was made by N. Schoubye (20). In B deficiency, highest alkalinity was found in the caecum, but the contrasts with normals were not striking. A similar exploration by McCarrison (21) which was extended to various organs showed a significant increase in acidity in the ventricle of the heart and in the brain.

Consideration has also been given to abnormalities of the digestive enzymes in thiamin deficiency. Peptic digestion is normal (22); so is the tryptic and ereptic function (23). It will be convenient at this point to refer also to certain enzymatic disturbances which are reflected in the blood or tissues, as well as to those peculiar to the digestive tract. The following tabulation is taken from an extended study on rats (24).

INFLUENCE OF VITAMIN B DEFICIENCY ON THE CONCENTRATION OF BLOOD AND TISSUE ENZYMES

Enzyme	Per Cent Change from Normal	
	Decrease	Increase
Blood serum amylase	12.4	—
Blood serum esterase	9.1	—
Blood serum phosphatase	—	12.5
Kidney phosphatase	14.4	—
Pancreatic amylase	0.0	0.0
Pancreatic lipase	23.0	—
Hepatic lipase	20.5	—
Pancreatic esterase	43.7	—
Hepatic esterase	6.4	—

The authors referred to do not regard the changes in proteolytic or amylolytic enzymes as very significant; the decreases in pancreatic and hepatic lipases, however, are held to suggest a disturbance in the digestion of fats. The study also included the effect of B complex deficiency on enzyme concentration. An increase in the blood serum phosphatase was noted in this condition, while deficiency of thermostable factors alone led to a 20 per cent decrease in the concentration of this enzyme. Otherwise, multiple deficiencies of the B factors did not appear to produce significant changes. Nor did combination of vitamin A and B complex deficiencies alter the effects of the separate deficiencies (25). Incidentally, large amounts of vitamin A do not affect the thiamin requirement of growing rats (26). A result,

conflicting in part with the findings of Sure *et al.*, is to the effect that pancreatic esterase and hepatic amylase are increased in beriberic animals (27). The deaminase activity of polyneuritic heart tissue is reported as subnormal (28).

Blood Chemistry

The most marked effect of thiamin deficiency upon blood chemistry is anhydremia (29), as evidenced by increase of serum proteins. This is accompanied, according to this study, by hypoglycemia in nursing rats, a fall in hemoglobin and erythrocyte count, and a general deterioration in the blood regenerating function. Non-protein nitrogen was not altered (30). Other evidence suggests that the blood regenerative factor is not thiamin but another and thermostable factor (31). Also Salmon and Goodman (32), while confirming the anhydremia in rats as well as in pigeons, found a high hemoglobin content. However, since these authors observed the same peculiarity in rats on sparing amounts of a thiamin-adequate diet, the effect is attributed to inanition.

Several other aspects of blood chemistry are considered for convenience later in this chapter in connection with methyl glyoxal, lipids, etc. Others have been considered in connection with glycogen and blood sugar in Chapter XXV. Galamini (33) has noted a longer persistence of alcohol in the blood of beriberic pigeons than in that of normal ones. Tisolowitz (34) notes a lowered rate of disappearance of congo red from the blood stream upon administration of thiamin, which he regards as producing a reduction of permeability of the walls of the vessels.

Among the endocrine glands affected by B_1 deficiency the adrenals are prominent. They show hypertrophy, especially of the medulla. Nagai (35) notes an increased coenzyme content of the adrenals in thiamin deficiency and attributes a beneficial effect of adrenal cortex feeding in this deficiency to its coenzyme content. An increased secretion of adrenalin has been reported by several workers but has been denied by others (36). The chemical facts are not clear. This is equally true of the thymus, the thyroid, parathyroids, testes, ovaries, and spleen, which undergo anatomical or functional changes which have been attributed to B_1 deficiency, but which have not been analyzed chemically. Most of the studies of the endocrines which have been made in this connection belong to an era of exploration for significant clues to the principal role of vitamin B_1. In this sense they may be regarded as negative, although future research with more modern methods may well reveal useful and significant relationships.

Somewhat more positive results have been obtained in studies of thyroxine in relation to vitamin B_1. Hyperthyroidism was induced by Cowgill (37) in dogs on a normal diet by feeding thyroid gland. As a result they exhibited a marked loss of appetite which could be corrected by giving a vitamin B_1 concentrate. During hyperthyroidism accompanied by a liberal B_1 supply, the voluntary consumption of food rose to twice that of dogs not receiving thyroid. Likewise in pigeons, Cowgill found a rise in the B_1 requirement in hyperthyroidism. Smith and Sure have obtained somewhat similar results, *i.e.*, that there is a balance between thyroxin and thiamin so that the latter can afford protection against hyperthyroidism (38). There is no evidence, however, of hyperthyroidism as a consequence of B_1 deficiency. The antagonism between thyroxine and thiamin has been confirmed by studies involving synthetic thiamin (39). Doses up to 100 γ daily of thiamin counteract the toxicity of as high a daily dose as 0.2 mg. of thyroxin.

Several peculiarities of varied character which have been noted in thiamin-deficient rats may well be recorded here. Smith, McClosky, and Hendrick (40) noted increased susceptibility to pilocarpine and ergotoxine. Tosatti (41) observed a decreased resistance to anaphylactic shock and to histamine shock. Baglioni and Console (42) are at variance with Kauffman-Cosla and Oeriu (43), the former authors maintaining that injection of insulin retards the onset of polyneuritis in pigeons as does also Shinobe (44). An extension of the lives of pigeons on a polished rice diet by administration of folliculin and other phenanthrene substances is claimed (45), but the results do not appear to be very consistent or impressive. Molitor and Sampson (46) found no specific effect of thiamin on the response of rats to thujone. Convulsions were produced by equal doses. Inanition, attendant upon thiamin deficiency, however, increased the sensitivity to thujone.

Mineral Metabolism

The bones of birds fed on polished rice were found lighter than normal (47), which, however, might well be due to the mineral deficiencies of the diet. Special attention has been called to this feature by McHargue and Roy (48), as they believe pigeons are protected to some extent from polyneuritis by adequate supplies of the mineral elements in the food. Calcium content of the blood of polyneuritic pigeons has been reported normal (49). The elimination of this element is said to be unchanged in B_1 deficiency in dogs (50), but Miyadera (51) differs in finding that assimilation is increased. Uga (52) reports beriberic human milk low in calcium. Marked increase in potassium

in the skin, occasional increase of calcium, more often increase of magnesium, a rise in chlorides and a fall in phosphates represent the observations of Fawns and Jung (53). Oh (54) found low blood calcium in human beriberics and reported benefit from treatment with calcium preparations.

Similarly conflicting results are recorded concerning phosphorus. Schmitz and Hiraoka (55) believe a low phosphatide content of blood and tissues to be prevalent in B_1 avitaminosis of pigeons, but little general support is found elsewhere for the idea. Nagai has studied the distribution of organic and inorganic forms of phosphorus in B_1 deficiency (56). The total inorganic and acid-soluble phosphorus is not greatly changed in the rat or pigeon, although the relative quantities in the several tissues are somewhat increased or decreased. Hexose monophosphate and adenylic acid are said to be increased in muscles. The phosphatese and phosphatase activities are increased in some organs and diminished in others, but in a way that does not offer any very definite suggestion as to the significance of the findings. Certainly there is no conspicuous defect in mineral metabolism in beriberi as is the case in rickets.

Glutathione

Randoin and Fabre compared the tissues of B_1 deficient pigeons with those of birds which were underfed on a diet adequate in B_1. The glutathione content was diminished in all tissues in the B_1 deficient birds, most of all in the musculature. This deficiency developed as the weight and body temperature began to fall (57). Like studies were reported by di Mattei (58), but the deficiencies of glutathione content were scarcely sufficient to be of significance. Nagai (59) found rather substantial reduction of glutathione content, especially in heart, bone marrow, and muscle, but not in the liver, where it appeared to be increased.

A deficiency of glutathione in beriberic tissues is of considerable interest in connection with the occurrence of methyl glyoxal in the blood and urine of beriberic human beings. Peters (60) offers the suggestion that the presence of methyl glyoxal may be due to the deficiency of glutathione, which is known to function as a coenzyme for glyoxalase, the enzyme which converts glyoxal to lactic acid:

$$CH_3COCHO + H_2O \longrightarrow CH_3CHOHCOOH$$

Hirano (61) reports recovery from convulsions in pigeons fed upon polished rice following injection of glutathione, but life presently terminated if the birds were supplied with glutathione without the vitamin.

Methyl Glyoxal

Glyoxal is merely pyruvic aldehyde and chemically represents a stage of oxidation less advanced by one step than pyruvic acid. In contrast with lactic acid, methyl glyoxal represents incomplete oxidation of the terminal carbon atom, while in lactic it is the central carbon atom which is incompletely oxidized. Its origin and fate in the body are uncertain. Whether or not methyl glyoxal is an intermediary in normal carbohydrate metabolism is still under debate. Formerly it was widely regarded as an essential intermediate, but since the rise of the Embden-Meyerhof scheme this idea has lost in favor.

The occurrence of methyl glyoxal in human beriberi has been strongly emphasized by a large group of Japanese workers, who have published their results in the *Tohoku Journal of Experimental Medicine*. According to these writers this substance occurs in copious amounts as an active toxin in the blood and in the secretions of human beriberics. These findings and numerous others are contained in an extended series of some seventy-five articles appearing in this Journal over a period of fifteen years or more. These appear to have been ignored or overlooked by Western scientists generally. Since the papers are often fragmentary, somewhat repetitious, and inconclusive, detailed references will be omitted, but attention is called to the enormous volume of experimental work and the vast amount of clinical material involving hundreds of human beriberi cases which are recorded in some of them, *e.g.*, Chiba (62). The principal findings will be mentioned at this point: Breast milk of beriberic women lacks a peroxidase present in normal milk. Peroxidase-negative milk is also secreted by experimental animals upon thiamin-deficient diets (65). The milk does not become peroxidase positive on injection of thiamin unless accompanied by a liver substance to which the name "Yakriton" is given. As the milk becomes positive, the young begin to thrive (64). Mice fed on polished rice with daily injection of human or rabbit milk die much more quickly with characteristic symptoms of polyneuritis if the injected milk is peroxidase negative. Deaths may be induced in one to five days by this means. If thiamin is administered, the milk loses its toxic potency, although it still remains peroxidase negative (65). In women one breast may secrete peroxidase-positive and the other peroxidase-negative milk (66). The blood leucocytes of beriberics, whether of humans or of experimental animals, exhibit a deficiency of peroxidase as ascertained by a copper test. This deficiency disappears in thiamin treatment of pigeons (67).

Methyl glyoxal has not been wholly overlooked by the Western world. Vogt-Møller (68) noted that this substance appears in a

liver-extract-hexosephosphate medium when the liver involved is obtained from B_1 deficient animals. Thiamin-deficient animals cured by supplying the deficient vitamin yield liver extracts which produce no glyoxal. Vogt-Møller believes that the responsible fact is the absence of a coenzyme, since glyoxal disappears on adding to the system a boiled extract from a normal liver.

Geiger and Rosenberg (69) found methyl glyoxal in the urine of thiamin-deficient infants. Peters comments in this connection that the methyl glyoxal theory of carbohydrate degradation which has not been in favor lately may be necessary to an adequate explanation (70). According to Peters methyl glyoxal is entirely absent in the brains of polyneuritic pigeons.

Lehmann (71) found methyl glyoxal to be somewhat higher in the urine of B_1 deficient rats than in normal ones, but present in both. Jansen and Westenbrink (72) found polyneuritic rat liver unimpaired as to the conversion of methyl glyoxal to lactic acid and consider it improbable that polyneuritis is an intoxication by this substance. The substance was isolated in substantial amounts as the 2,4-dinitrophenylhydrazone from the blood of human beriberics, and the blood content of it was found to fall to normal after administration of thiamin (73). Aryama (74) observed no effect by venous injection of 0.5 gm. of methyl glyoxal per kilo of body weight in rabbits, nor, under the conditions observed, did the oral administration of the substance accelerate polyneuritis in pigeons on a B_1 deficient diet. Takamatsu, however, reported young rabbits dying with enlarged hearts from the injection of phenyl glyoxal when the diet was deficient in thiamin but not otherwise. Dilation of the mouse heart was also found under similar conditions.

In summary it may be said that the accumulation of pyruvic acid is a consequence of B_1 deficiency and appears to be supported by somewhat better experimental evidence than is available with regard to methyl glyoxal. Nevertheless, the work of the Japanese investigators is impressive. Further investigation is required to determine whether it is a direct consequence of thiamin deficiency or of some concomitant deficiency common in human beriberi. That some such substance as methyl glyoxal is a modifying causative agent of acute wet beriberi, particularly in infants, seems an attractive and plausible theory. We have long needed some rational explanation of the distinction between the dry and wet types.

Lipide Metabolism

One could continue almost interminably reciting abnormalities of physiological chemistry alleged to be associated with thiamin deficiency and oppose these allegations with equally

satisfactory citations in a contrary sense. No good purpose would be served thereby, as it must be evident from what has been said that the effects of thiamin deficiency are so many and so varied that it is impossible at present to segregate the primary consequences from the secondary ones or to distinguish which are due to thiamin deficiency and which to other concomitant deficiencies.

We shall, however, in closing discuss briefly the subject of lipide metabolism in this connection. Cholesterol of the brain of normal and polyneuritic animals was studied by Westenbrink (76), who also reviewed the previous literature carefully. He could not find a significant difference attributable to avitaminosis. Cholesterol in the blood of polyneuritic animals has been reported as normal in the incipient stages, rising in intermediate stages above normal and again falling to normal during the development of acute symptoms (77).

Monaghan (78) could find no evidence for any connection between thiamin and phospholipid metabolism. The phospholipid contents of tissues of rats, normal or avitaminotic, are reported very constant. Sure and associates (79) likewise could find no changes in the blood fatty acids or blood cholesterol in rats. Absence of any relationship between avitaminosis and total cholesterol, phosphatide, phosphorus, or amino nitrogen of the lipide material of the brain could be found in pigeons according to Takata (80). He did, however, find a decrease in polyneuritis of the total fatty acid content of the brain, in the total lipide content of the spinal cord, and in the fatty acid content of the peripheral nerves. This author regards thiamin as inhibiting the oxidation of fat in the nerve tissue. Perhaps there is a connection between this observation and the apparently more positive indications discussed in Chapter XXVI that thiamin function may have to do with the synthesis of fats from carbohydrates.

Summary

Aside from the disturbances of carbohydrate metabolism discussed in a previous chapter, the biochemical upset associated with lack of thiamin is widespread and profound, but intangible as to precise cause. The evidence is confused and often contradictory.

BIBLIOGRAPHY

1. Graham, C. E., and Griffith, W. H.: Effect of Vitamins B and G on the Consumption and Utilization of Food. J. Nutrition *6*, 195, 1933.
2. Harris, L. J.: The Vitamin B_2 Complex. Differentiation of the Antiblacktongue and P-P Factors from Lactoflavin and Vitamin B_6. Biochem. J. *31*, 1414, 1937.
3. Sure, B.: A Detailed Study of the Role of Vitamin B_1 in Anorexia in the Albino Rat. J. Nutrition *1*, 49, 1928.
4. Cowgill, G. R.: Vitamin B Requirement of Man. Yale Univ. Press, 1934, pp. 25–28.
5. Harris, L. J., Clay, J., Hargreaves, F. J., and Ward, A.: Appetite and Choice of Diet: Ability of the Vitamin B Deficient Rat to Discriminate between Diets Containing and Lacking the Vitamin. Proc. Roy. Soc. Lond. *B 113*, 161, 1933.
6. Wright, S.: The Effect of Vitamin B on the Appetite. Lancet II, 1207, 1921. Vitamin B and the Alimentary Canal. Lancet I, 263, 1928.
7. Sparks, M. I., and Collins, E. N.: Role of Vitamin B_1 in Tonus of Large Intestine. Amer. Jour. Digest. Dis. and Nutrition *2*, 618, 1935.
8. Moore, C. N., and Plymate, H. B.: The B Vitamins. VI. Pyloric Obstruction in Rats. Amer. Jour. Physiol. *102*, 605, 1933.
9. Dalldorf, G., and Kellogg, M.: Incidence of Gastric Ulcer in Albino Rats Fed Diets Deficient in Vitamin B_1. Jour. Exp. Med. *56*, 391, 1932.
10. Simpson, G. K.: Observations upon Gastritis. Gastric Lesions of Vitamin B_1 Deficiency in Albino Rat. Guy's Hosp. Rept. *86*, 120, 1936.
11. Eddy, W. H., and Dalldorf, G.: The Avitaminoses. Williams and Wilkins, Baltimore, 1937.
12. Westenbrink, H. G. K., and Overbeek, G. A.: Vitamin B und Darmresorption. Arch. Neerl. Physiol. *18*, 416, 1933.
13. Webster, D. R., and Armour, J. C.: Effect of Vitamin Deficiency on Gastric Secretion. Proc. Soc. Exp. Biol. Med. *30*, 1297, 1933.
14. Farnum, M. B.: Gastric Secretion in Experimental Beriberi in the Dog. Arch. Int. Med. *37*, 212, 1926.
15. Skarzynska-Gutowska, M.: Action Physiologique de la Vitamine B. Phénomènes de Sécretion de Quelques Glandes. Comp. Rend. Soc. Biol. *99*, 1168, 1928.
16. Ohta, K.: Zur Klinik der Säuglings-Beriberi. Jahr. f. Kinderheilk. *128*, 1, 1930.
17. Joffe, P. M., and Jolliffe, N.: The Gastric Acidity in Alcohol Addicts. Amer. Jour. Med. Sci. *193*, 501, 1937.

18. Alvarez, W. C., Pilcher, F., Foley, M. A., and Mayer, A.: Effects on Gastric Juice of Man of Six Weeks' Deprivation of Vitamin B_1, Amer. Jour. Digest. Dis. and Nutrition *3*, 102, 1936.
19. Masuko, R.: Digestive Juice Secretion on Diets Containing Rice of Varying Degrees of Refinement. Aichi Igakkai Zasshi. *42*, 1721, 1935.
20. Schoubye, N.: Researches into the H-ion Concentration in Different Segments of the Intestinal Canal in Animals on Normal and Vitamin B-free Diets. Hospitalstidende *71*, 201, 1928. Studies on H-ion Concentration in Different Regions of the Intestinal Canal, etc. Acta. Med. Scand. Suppl. *26*, 537, 1928.
21. McCarrison, R.: *p*H of Organs in Normal and Pathological Conditions. Current Sci. *1*, 8, 1932.
22. Sure, B., and Harrelson, R. T.: Enzymic Efficiency in Avitaminosis. VII. Peptic Digestion in Vitamin B Deficiency. Amer. Jour. Digest. Dis. and Nutrition *4*, 177, 1937.
23. Sure, B., Kik, M. C., and Buchanan, K. S.: Enzymatic Efficiency in Avitaminosis. I. Influence of Vitamin B Deficiency on Tryptic and Ereptic Digestion of Casein. Jour. Biol. Chem. *108*, 19, 1935.
24. Kik, M. C., Sure, B., Buchanan, K. S., and De Witt, J.: Enzymic Efficiency in Avitaminosis; Influence of Vitamin B_1 and G Deficiencies on Concentration of Blood and Tissue Enzymes. Amer. Jour. Digest. Dis. and Nutrition *3*, 490, 1936.
25. Sure, B., and Buchanan, K. S.: Effect of Deficiency of Vitamin A and B Complex on Concentration of Blood and Tissue Enzymes of the Albino Rat. Proc. Soc. Exp. Biol. Med. *35*, 210, 1936.
26. Scheunert, A., and Rau, S.: Über den Einfluss verschiedener Vitamin A Mengen auf den Vitamin B_1-Bedarf. I. Mitteilung zum Synergismus der Vitamine. Z. physiol. Chem. *246*, 267, 1937.
27. Tsunoo, S., Takamatsu, M., Kamachi, T., and Inaizumi, M.: Enzyme Action in the Avian Organism during B-Avitaminosis. J. Biochem. (Japan) *22*, 225, 1935.
28. Birch, T. W., and Mapson, L. W.: Role of Adenylic Acid in Vitamin B_1 Deficiency. Nature *138*, 27, 1936.
29. Sure, B., and Smith, M. E.: Effect of Vitamin Deficiencies in Carbohydrate Metabolism. Jour. Biol. Chem. *82*, 307, 1929.
30. Sure, B., and Kik, M. C.: Avitaminosis. VIII. Effect of Vitamin B Deficiency on Concentration of Non-protein Nitrogen of Blood. Proc. Soc. Exp. Biol. Med. *29*, 462, 1932.
31. Wills, L.: Nature of Hemapoietic Factor in Marmite (Yeast Extract). Lancet I, 1283, 1933.
32. Salmon, W. D., and Goodman, J. G.: Relation of Vitamin B to Hemoglobin Content of Blood. Ala. Agr. Exp. Sta. Ann. Rept. *43*, 20, 1932.
33. Galamini, A.: Blood-alcohol Curve and Experimental Beriberi. Atti. Accad. Lincei. *23*, 623, 1936.

34. Tisolowitz, R.: Wirkung der Vitamins B_1 und C auf die Dauer des Verbleibens von Kongorot in der Blutbahn. Biochem. Zeit. *291*, 70, 1937.
35. Nagai, S.: Coenzyme Content of Suprarenals of Chickens with Avitaminosis. Jour. Biochem. (Japan) *16*, 383, 1932.
36. Marrian, G. F., Baker, L. C., Drummond, J. C., and Woollard, H. H.: The Physiological Role of Vitamin B. V. The Relation of Inanition to Vitamin B Deficiency in the Pigeon. Biochem. J. *21*, 1336, 1927.
37. Cowgill, G. R.: Vitamin B Requirement of Man. Yale Univ. Press, 1934.
38. Sure, B., and Smith, M. E.: Hyperthyroidism and Nutrition. Vitamin B and Thyroxine. J. Nutrition *7*, 547, 1934.
39. Sure, B., and Buchanan, K. S.: Antithyrogenic Action of Crystalline Vitamin B. J. Nutrition *13*, 513, 1937.
40. Smith, M. I., McClosky, W. T., and Hendrick, E. G.: The Influence of Vitamin Deficiencies on Susceptibility to Certain Poisons. U. S. Public Health Reports *41*, 767, 1926.
41. Tosatti, E.: Anaphylactic Shock in Beriberic Pigeons. Boll. Soc. Ital. biol. sper. *8*, 1732, 1933.
42. Baglioni, A., and Console, J.: Insulin and Experimental Beriberi in the Pigeon. Boll. Soc. Ital. biol. sper. *9*, 153, 1934.
43. Kauffman-Cosla, O., and Oeriu, S.: Die Wirkung des Insulins auf experimentelle Beri-Beri und experimenteller Avitaminose B. Arch. f. exper. Path. u. Pharm. *170*, 458, 1933.
44. Shinobe, S.: Studies on the Influence of Various Glands of Internal Secretion on the Gas Exchange in Animals Suffering from B Avitaminosis. II. The Influence of Insulin. Folia endocrin. Japan *7*, 40, 1931.
45. Sanchez-Rodrigues, J., and Morros Sarda, J.: Antiberiberiwirkung der Phenanthrensubstanzen. Z. Vitaminforsch. *6*, 193, 1937.
46. Molitor, H., and Sampson, W L.: Über die Wirkungen hoher Gaben von natürlichen und synthetischem Vitamin B_1. E. Merck's Jahresbericht, p. 51, 1936.
47. Findlay, G. M.: An Experimental Study of Avian Beriberi. Jour. Path. Bact. *24*, 175, 1921.
48. McHargue, J. S., and Roy, W. R.: The Effect of a Diet of Polished Rice on the Mineral Content of the Carcasses of Pigeons. Amer. Jour. Physiol. *99*, 221, 1931.
49. Ungar, A.: Über den Ca-Gehalt des Blutserums bei Mangel an Vitamin B. Biochem. Z. *180*, 357, 1927.
50. Palladin, A., and Kudrjawzewa, A.: Beiträge zur Biochemie der Avitaminosen. 5. Untersuchungen über den Stoffwechsel bei avitaminös ernährten Kaninchen. Biochem. Z. *154*, 104, 1924.
51. Miyadera, K.: Über die Beziehungen der Vitaminfunktion und Kalkstoffwechsel. Biochem. Z. *124*, 244, 1922.

52. Uga, Y.: The Peroxidase Reaction. LIII. The Arakawa Reaction and the Calcium Content of Human Milk. Tohoku Jour. Exp. Med. *25,* 169, 1935.
53. Fawns, H. T., and Jung, A.: The Mineral Content of the Skin of Rats Suffering from Beriberi, with Investigations on the Formation of Vitamin B_1 by Molds. Biochem. J. *27,* 918, 1933.
54. Oh, H. Y.: Etiology of Beriberi. Korean Med. Jour. *2,* 113, 1932.
55. Schmitz, E., and Hiraoka, T.: Über den Phosphatidstoffwechsel bei der B-avitaminose der Tauben. Biochem. Z. *193,* 1, 1928.
56. Nagai, S.: The Phosphates in Muscles in B Avitaminosis. J. Biochem. (Japan) *16,* 351, 1932.
57. Randoin, L., and Fabre, R.: Recherches Comparatives sur la Teneur en Glutathion de Quelques Tissues et du Sang, chez le Pigeon Normal, chez le Pigeon Sous-ailmente, et chez le Pigeon Privé de Vitamines B. Compt. Rend. *185,* 1515, 1927.
58. Di Mattei, P.: Tissue Glutathione in Avian Beriberi. Biochem. e. Terap. Sperim. *15,* 366, 1928.
59. Nagai, S.: Glutathione Content of Tissues in Avitaminosis. J. Biochem. (Japan) *16,* 371, 1932.
60. Peters, R. A.: Vitamin B_1 (Oryzanin, Torulin, Aneurin). Its Chemistry and Mode of Action. Current. Sci. *5,* 207, 1936.
61. Hirano, Y.: Influence of Glutathione on Polyneuritis of Pigeons Fed with Polished Rice. Orient J. Dis. Infants *17,* 8, 1935.
62. Chiba, M.: Peroxidase Reaction. XLI. Prolongation of Short-timed Peroxidase Reaction of Leucocytes as a Sign of Avitaminosis B and Effect of Vitamin B on It. A Clinical Experiment. Tohoku J. Exp. Med. *19,* 486, 1932.
63. Arakawa, T.: The Peroxidase Reaction. XXVIII, XXIX, XXX, and XXXI. An Exceedingly Sensitive Peroxidase Reagent for Human Milk. Tohoku J. Exp. Med. *16,* 83 *et seq.,* 1930.
Arakawa, T.: The Peroxidase Reaction. XXXIII. Biological Significance of Milk Peroxidase. Preliminary Report Concerning a Relation between Vitamin B and Peroxidase. Tohoku J. Exp. Med. *19,* 178, 1932.
Suzuki, K., and Arakawa, T.: The Peroxidase Reaction. XXXIV. "Aperoxidatic" Milk as a Possibly Early Symptom of Avitaminosis B. Tohoku J. Exp. Med. *19,* 183, 1932.
64. Asakura, K.: The Detoxicating Hormone of the Liver (Yakriton). XXXVII. The Effect of Yakriton on a Negative Arakawa Reaction. Tohoku Jour. Exp. Med. *19,* 269, 1932.
65. Asakura, K., and Ohsako, H.: The Peroxidase Reaction. XLII. Arakawa's Reaction and Toxicity of Human Milk. Tohoku J. Exp. Med. *20,* 429, 1933.
66. Asakura, K.: Peroxidase Reaction. XXXVIII. Relation between Arakawa's Reaction and Suitability of Human Milk. Tohoku J. Exp. Med. *19,* 275, 1932.
67. Chiba, M.: Peroxidase Reaction. XXXIX. Short-timed Peroxi-

dase Reaction of Blood Leucocytes of B-avitaminotic Pigeons. Tohoku J. Exp. Med. *19*, 282, 1932.

68. Vogt-Møller, P.: Ist Avitaminosis B_1 eine Intoxikation mit Methylglyoxal? Biochem. Z. *233*, 248, 1931.
69. Geiger, A., and Rosenberg, A.: Methylglyoxal im Harne und in der Cerebrospinalflüssigkeit bei Ernährungsstörungen der Säuglinge mit toxischen Symptomen und bei der experimentellen B_1-avitaminose bei Hunden und Ratten. Klin. Wochschr. *12*, 1258, 1933.
70. Gaddie, R., and Stewart, C. P.: The role of glutathione in muscle glycolysis. Biochem. J. *29*, 2101, 1935.
71. Lehmann, J.: Ist das Vorkommen von Methylglyoxal in Harn specifisch für B_1-Avitaminose? Skand. Arch. Physiol. *71*, 157, 1935.
72. Jansen, B. C. P., and Westenbrink, H. G. K.: Is beriberi an intoxication by methylglyoxal? Acta Brevia. Neerland *2*, 1, 1932.
73. Shindo, T.: Die bisulfitbindende Substanz im Menschenblute bei Beriberi. Zeit. physiol. Chem. *247*, III, 1937.
74. Ariyama, Tsune.: The Products of Decomposition of Sugar by Beriberi Bacteria. Isolation of Methylglyoxal and Its Physiological Actions. Jour. Agri. Chem. Soc. Japan *7*, 763, 1931.
75. Takamatsu, A.: Heart-enlarging Effect of Phenylglyoxal. LXI. Peroxidase Reaction. Tohoku J. Exp. Med. *27*, 434, 1935.
76. Westenbrink, H. G. K.: Über den Gehalt an freiem und gebundenem Cholesterin verschiedener Teile des Gehirns von normalen und Polyneuritis-Tieren. Arch. Neerl. Physiol. *19*, 122, 1934.
77. Bruck, A.: Dynamik des Cholesterins im Blute von Tauben im Entwicklungsprocess der B-Avitaminose. Zeit. f. Vitaminforsch. *6*, 289, 1937.
78. Monaghan, B. R.: The Effect of Dietary Deficiencies on Phospholipide Metabolism. Jour. Biol. Chem. *98*, 21, 1932.
79. Sure, B., Kik, M. C., and Church, A. E.: Avitaminosis. X. Further Studies on the Effect of Vitamin B Deficiency on Lipid Metabolism. Proc. Soc. Exp. Biol. and Med. *29*, 849, 1932.
80. Takata, Y.: Fat Metabolism in the Nerve Tissues of Pigeons Affected by B Avitaminosis. J. Biochem. (Japan) *24*, 153, 1936.

CHAPTER XXVIII

THE GENERAL ROLE OF THIAMIN IN LIVING THINGS

A CONTROVERSY which took place seventy to eighty years ago between Liebig and Pasteur marks the origin of the question as to whether microorganisms need for their proper growth preformed supplies of certain specific substances of the category that we now denominate as vitamins, or, somewhat more explicitly, nutrilites (1). Since Pasteur's time, an affirmative answer has been given. The ramifications of such studies have been so extensive that several months of tireless reading would be required to attain a mastery of all that has been published on the subject. A limited number of findings now lie within the borders of the proven; a much larger number are reasonable inferences; many of the broader generalizations are still of the nature of plausible surmises.

The obstacles to rapid progress have been of three principal sorts: (1) The difficulty of isolating nutrilites or supposed nutrilites in pure form for study of their effects; (2) the fact that the requirements of a given organism have in very many instances proved to be multiple, that is, to involve some two or more specific substances, often occurring mixed in varying proportions in extracts of natural tissues, and (3) symbiosis, whereby an organism is able to exist only by virtue of the synthetic activities of another whose presence is often easily overlooked or appears inconsequential.

Among the substances which are regarded as functioning as nutrilites are thiamin, riboflavin, vitamin C, biotin, pantothenic acid, nicotinamide, auxin a, auxin b, hetero-auxin (β indolylacetic acid), inositol, folliculin, etc. These differ with respect to the diversity of organisms in which they are supposed to function, with respect to their quantitative biopotency, and with respect to degree of indispensability. Some are not yet isolated and therefore cannot be regarded as fully established entities. The evidence is as yet incomplete but the events of recent years appear to justify the supposition that many, if not all, the vitamins and hormones utilized by animals also play some role in plants and lower organisms. Probably we shall find some whose

role in the animal world is important which play only a minor part in plants or vice versa. Indeed it is not unlikely that some will prove of little consequence to certain groups of plants though important for others, just as we have already found that a deficiency of certain of the vitamins is not very detrimental to some species of animals. For example, the rat does not need an external supply of vitamin C and pigeons show little or no susceptibility to a lack of vitamin B_2 in the adult stage of life.

However, in speaking of the needs of an organism we have learned to be judicious in our language, as the world "requirement" can be used properly in two different senses. It may refer to a supply needed from an external source or to need in a broader sense without regard to the origin of the substance. In the first sense the rat does not need vitamin C because it can synthesize its own supply. In the second sense the rat does need C, for the substance is found in its organs and clearly enters into its physiological processes. In general, in the animal world we refer to the endogenous accessory substances as hormones and to the exogenous ones as vitamins. If the majority of animals were like the rat in this respect, we should have classified ascorbic acid as a hormone.

The distinction is even more necessary in relation to plants and microorganisms than it is for animals, at least at the present stage of progress. Some nutrilites can be synthesized by certain plants and not by others. The latter depend upon the former or upon animal life for their supply of the particular nutrilite or nutrilites which they are unable to synthesize. Those which can synthesize a particular nutrilite are called autotrophic with respect to it; those which cannot are called heterotrophic. It is believed that higher plants are in general autotrophic for most of their growth factors. The saprophytic plants such as the molds and fungi appear to be more often heterotrophic with respect to growth factors. Microorganisms evidently present considerable variety but perhaps may best be thought of as autotrophism. Many organisms have been found which are occupying an intermediate position.

A further precaution is necessary, for there are degrees of autotrophism for a growth factor to the extent that they can make a slow growth without an external supply but nevertheless grow more vigorously with an external supply. Presumably such organisms have not yet evolved full synthetic powers or by reason of their saprophytic habits have degenerated and lost them. Furthermore, a plant may be able to synthesize its own nutrilite in a particular case, at one stage of its life cycle but not at another, or it may be able to do so in one of its parts and not in another. Accordingly, an economy arises involving storage of

the nutrilite for future use or its transport from one part to another.

Thiamin appears to function in all these ways for different lower organisms. Since it is indispensable for higher animals, for insects, and for higher plants, it surpasses all other known specific substances in the scope of its biochemical significance. Riboflavin is its nearest competitor for this distinction among the substances of known constitution. Biotin ranks very high in importance in the plant world (2), but little is known of its chemical nature. Although thiamin has been available in pure form for only a few years, already a vast amount of data has accumulated regarding its occurrence and operation in lower as well as higher organisms. We shall confine our further discussion of nutrilites to it. The reader is referred elsewhere (3) for a more general treatment of the subject. An excellent recent discussion is that of Bonner (4).

We have given above a sketch in perspective of the general subject. However, in doing so we have made many inferences that cannot yet be said to represent proved truths. Obviously, what is true of a certain selection of species may not prove general when further species are examined. Equally, what is true of one nutrilite or growth factor may prove to be exceptional rather than typical. It will therefore be well to give a résumé of the evidence regarding thiamin.

Thiamin and Yeast Growth

In 1901 Wildiers made the observation that the yeast which he was studying was unable to grow appreciably from a small inoculum in synthetic media. He postulated an essential growth substance in the natural media which he called "bios." His characterization of bios was such as to suggest to R. J. Williams the possible identity of bios and vitamin B. Accordingly, it was proposed to utilize the growth of yeast as a test for vitamin B (5, 6), a proposal which never reached fruition but which provoked a great deal of investigation and some controversy. The upshot was that various strains of yeast were found to have quite diverse requirements. Among the yeast-growth stimulants found to be effective in various combinations with various strains were inositol (7, 8), vitamin B_1 (9), pantothenic acid (10, 11), biotin (12), as well as other substances which probably include some of the thermostable B vitamins found in yeast by animal experiment.

It has not been demonstrated conclusively whether or not yeast can grow at some extremely slow rate on a synthetic medium containing nothing of a nutrilite nature. It has been shown that even with small seedings of yeast, such that the

amounts of the previous medium carried over with the seed are reduced to extremely small quantities, yeast can make a slow growth on synthetic media (13). However, the seeding in such experiments has not been successfully reduced to a single cell, and it seems wholly probable to the present authors that even the slow growth of certain cells is at the expense of the death and decomposition of other cells of the seeding, or by a redistribution by diffusion of the meager nutrilite supply from one living cell to another. If so, it would be fair to conclude that no growth is possible without nutrilite supply. Whether or not this be true, it has been clearly shown that certain yeasts can synthesize substances which stimulate yeast growth, among them thiamin. However, there are surprising differences in the synthetic capacities as well as in the requirements of different strains of yeast. For all of them, normally rapid growth is possible only with an external supply of nutrilites. As far as thiamin is concerned, it is clear that this substance, as present in commercial yeast, originates primarily not in the synthetic activities of the yeasts, but in those of the higher plants whose tissues or fluids enter into the medium for yeast growth. The quantity of thiamin present in the final yeast depends on that preformed in the medium. The growth of yeast in many commercial media can be promoted greatly by additions of thiamin, and the thiamin content of the resulting yeast rises proportionately. The richest commercial yeasts are those obtained by interrupting the growth and taking off the crop well before complete exhaustion of the food supply of the medium is approached (14). Addition of thiamin to yeast cultures also powerfully increases the rate of alcoholic fermentation (15). This increase may be regarded as evidence of increased vigor of its metabolic processes.

The long existence of an equivocal situation regarding the role of thiamin in yeasts, which are the organisms which have been most extensively studied, justifies caution in making categorical statements regarding the role of thiamin in lower organisms generally. Practically speaking, however, thiamin (or its intermediates) is required by yeast for its normal performance, and a general view of lower organisms indicates that this statement applies to all life. Indeed all organisms appear to use thiamin, some synthesizing it for themselves, other deriving it from the activities of other forms of life (12). In one sense or another symbiosis is an almost universal phenomenon in living nature. In its strictest sense, symbiosis applies only to two or more distinct organisms growing side by side in the same medium and exhibiting a certain degree of interdependence of bioactivity. However, it does not represent a great stretch of language to say that animal forms of life in general are in

symbiosis with plant forms, since they ultimately depend on the latter for food supply. In the case of microorganisms we cannot cultivate each individual in a separate environment. They all grow together, competing for the nutrient components and sharing and exchanging them. If the weaker individuals die and contribute the products of their synthetic activities to the perpetuation of the survivors, we have a sort of cannibalistic symbiosis. This seems to be the actual situation.

In our further discussion of the subject we will refer first to those organisms which have been shown to contain thiamin and therefore presumably have a use for it, and second to those organisms which have been demonstrated to need it or benefit by its presence in the medium.

Organisms Containing Thiamin

Higher plants. First we must refer to the long list of substances referred to in tables of foodstuffs in Chapter XVIII. These and hundreds of other plant and animal tissues have been assayed at least qualitatively for thiamin. While some contain very small amounts, it cannot be said that any of the natural tissues used as human food are quite devoid of the substance. Since all animal species which have been studied require a thiamin supply in their food (a few cases of synthesis by intestinal flora excepted), we must regard the plant world as the source of the substance. The quantities present in tissues are, however, very small, usually about one part in two million except in the vital parts of seeds. It would, therefore, be readily possible to overlook the presence of thiamin in tissues, especially if the quantities of tissues available for assay were small or the conditions of growth of the tissues tested were such as to diminish their thiamin content below normal.

The occurrence of thiamin is not limited to food plants, by all indications. Schopfer, who has made very extensive as well as very important contributions to the subject matter of this chapter, has assayed the leaves of 134 species of plants, utilizing the growth of the fungus Phycomyces as a test of the presence of thiamin (16). The medium used consisted of 10 per cent glucose, 0.1 per cent asparagin. 0.05 per cent magnesium sulfate, 0.15 per cent potassium acid phosphate. This medium had previously been proved incapable of supporting the normal development of this organism. If supplemented by minute traces of pure thiamin (0.002 to 0.005 γ per c.c. of medium), vigorous growth occurred on the medium (17) in proportion to the amount of thiamin added. Extensive further experience has increased the evidence of the specificity of this test for thiamin. The sole known substitute capable of producing an equal growth

of Phycomyces is an equimolecular mixture of the intermediates of thiamin, as we shall see below. Considerable faith can, therefore, be placed in a qualitative sense in the following tabulation of results taken from Schopfer's paper (16). The figure in the right-hand column indicates relative vigor of growth observed when a fragment of the indicated variety of leaf was sterilized with the medium before inoculating with the spores of Phycomyces. Inasmuch as unfavorable or toxic substances as well as thiamin must have been contributed to the medium by the leaf tissue, in certain cases, the fewer negative or nearly negative results cannot be regarded as final evidence of the absence of thiamin.

TABLE XXIV

Family	*Species*	*Growth*
Selaginellaceae	Selaginella sp.	4
Equisetaceae	Equisetum arvense	3
Polypodiaceae	Phyllitis Scolopendrium	3
	Cystopteris alpina	1
Ephedraceae	Ephedra sp.	3
Abietaceae	Picea exelsa	4
Betulaceae	Carpinus caroliniana	5
	Alnus incana	5
Moraceae	Ficus Carica	1
	Ficus nerifolia	1
	Morus nigra	4
Ulmaceae	Ulmus scabra	5
Piperaceae	Piper nigrum	2
Caryophyllaceae	Saponaria officinalis	4
	Melandrium album	5
	Silene vulgaris	4
Euphorbiaceae	Euphorbia Cyparissias	5
	Euphorbia sp.	3
Buxaceae	Pachysandra terminalis	5
Hamamelidaceae	Liquidambar styraciflua	2
Magnoliaceae	Liriodendron tulipifera	3
	Magnolia amabilis	3
Aristolochiaceae	Asarum europaeum	3
	Aristolochia tomentosa	5
Menispermaceae	Coccula laurifolia	3
Ranunculaceae	Aconitum Lycoctonum	3
Berberidaceae	Mahonia sp.	4
Nymphaeaceae	Nymphaea sp.	4
Papaveraceae	Papaver somniferum	3
	Papaver alpinum	3
	Chelidonium majus	0.5
Cruciferae	Brassica nigra	3
	Hesperis matronalis	2
Violaceae	Viola tricolor	5
Guttiferae	Hypericum perforatum	5
	Hypericum sp.	4
Crassulaceae	Sempervivum tectorum (flle. caulinaire)	3
	Sedum rupestre	1
Saxifragaceae	Saxifraga Aizoon	2
Rosaceae	Alchemilla vulgaris	3
	Prunus laurocerasus	2
	Spiraea sp.	3
	Prunus cerasifera	3
	Duchesnea indica	3

Family	*Species*	*Growth*
Rosaceae	Cotoneaster sp.	3
	Craetaegus orientalis	4
	Geum urbanum	4
	Sanguisorba officinalis	5
	Potentilla recta	5
Papilionaceae	Medicago lupulina	4
	Medicago sativa	5
	Onobrychis viciifolia	5
	Lotus corniculatus	5
	Trifolium pratense	4
	Phaseolus lunatus	5
Elaeagnaceae	Elaeagnus pungens	4
Myrtaceae	Calistemon sp.	3
Punicaceae	Punica Granatum	4
Oenotheraceae	Oenothera sp.	3
	Fuchsia sp.	5
	Trapa natans	3
Malvaceae	Abutilon sp.	4
	Althaea sp.	5
Linaceae	Linum flavum	3
Geraniaceae	Geranium silvaticum	2
Rutaceae	Ruta graveolens	0.5
	Citrus aurantium	3
	Citrus aurantium amara	5
Balsaminaceae	Impatiens parvifolia	4
Aquifoliaceae	Ilex Aquifolium	5
Vitaceae	Vitis vinifera	5
Cornaceae	Aucuba japonica	4
Araliaceae	Hedera Helix	4
Umbelliferae	Daucus Carota	5
	Heracleum Sphondylium	5
	Foeniculum officinale	4
Primulaceae	Primula vulgaris	?
	Lysimachia vulgaris	5
Ericaceae	Rhododendron hirsutum	0.5
	Andromeda sp.	1
Ebenaceae	Diospyros Lotus	5
Convolvulaceae	Convolvulus sepium	5
Cuscutaceae	Cuscuta (2 species)	4 and 5
Boraginaceae	Echium vulgare	1
	Borago officinalis	5
	Onosma scintillans	5
Solanaceae	Hyoscyamus niger	1
	Datura Stramonium	4
	Solanum tuberosum	5
Scrophulariaceae	Digitalis lutea	4
	Scrophularia nodosa	4
	Nicotiana Tabacum	4
	Linaria Cymbalaria	5
Orobanchaceae	Orobanche sp.	4
Labiatae	Monarda sp.	3
	Stachys officinalis	3
	Mentha verticillata	3
	Lamium album	3
	Prunella vulgaris	3
Plantaginaceae	Plantago lanceolata	5
Gentianaceae	Gentiana Cruciata	2
	Gentiana punctata	5
	Erythraea centaurium	3
Menyanthaceae	Menyanthes trifoliata	4
Apocynaceae	Vinca minor	4
Asclepiadaceae	Hoya carnosa	5
Oleaceae	Syringa vulgaris	5
	Fraxinus Ornus	4

Family	*Species*	*Growth*
Rubiaceae	Coffea arabica	3
Caprifoliaceae	Sambucus Nigra	5
	Viburnum sp.	3
Valerianaceae	Valeriana dioïca	2
Dipsacaceae	Scabiosa Columbaria	4
	Knautia silvatica	5
Cucurbitaceae	Cucurbita sp.	4
Campanulaceae	Campanula rapunculoïdes	5
Compositae	Centaurea Scabiosa	4
	Achillea Millefolium	5
	Artemisia Absinthium	5
	Cichorium Inthybus	3
	Taraxacum officinale	5
Hydrocharitaceae	Elodea canadensis	3–4
Liliaceae	Lilium croceum	4
	Phormium tenax	4
	Polygonatum umbellatum	5
	Yucca filamentosa	5
	Asparagus officinalis	4
Iridaceae	Iris pallida	4
Commelinaceae	Tradescantia sp.	4
Gramineae	Coix lacryma	4
	Zea Mays	4
	Arundinaria japonica	4
Orchidaceae	Stanhopea tigrina	5

Schopfer did not limit his studies to leaves but included flower parts, petals, anthers, and pollen. Brown dead leaves, as well as tissues lacking chlorophyll (*Cascuta, Orobanche*), were also tested, with positive results, and a few tissues were evaluated quantitatively. In the case of wheat germ, rice polish, milk, and various animal tissues (18) a good correspondence of the Phycomyces assay with the results of animal tests was observed. On the whole the evidence can leave no reasonable doubt that all living active tissues of higher plants contain thiamin.

Fungi. This class has not been studied much as to thiamin content. However, there is evidence of synthetic capacity for it in *Mucor* sp., *Zygorhynchus exponens, Absidia* sp., *Rhizopus nigricans, Thamnidium elegans, Chaetostylum Fresenii, Pilaira anomala, Aspergillus niger.*

Microorganisms. When we turn to bacteria and other microorganisms, the evidence is a little less clear, but on the whole points to a similar conclusion. We have commented (page 314) on the contribution to the thiamin supply of the cow, which is regularly made by its intestinal flora. Also, page 203, we have referred to the phenomenon of refection in rats as a sporadically disturbing element in the rat growth test for thiamin. These phenomena perhaps deserve some discussion at this point. In the former case the thiamin synthesis is attributed to *Flavobacterium vitarumen,* an organism which occurs constantly in the rumen of cattle, but which has also been cultivated quite successfully *in vitro.* In both cases it is capable of the synthesis of

vitamin B, including thiamin (19). In the instance of refection in the rat the synthetic activity has not been traced to a specific organism with certainty. Its principal locus of growth in the rat is the caecum, and it has been claimed that removal of the caecum prevents refection (20). However, W. H. Griffiths (21) states that synthesis may go on in other parts of the gut. The occurrence of refection in rats is irregular. It has never been observed in the present authors' laboratory, where many hundreds of rats were housed for years. Once started in a colony it is likely to spread, promoted, of course, by giving the rats access to the feces of refected animals. The use of raw starch in the diet promotes it. Indeed it appears to be associated rather definitely with some degree of impairment of starch digestion. In refected rats the caecal contents are acid, in normal rats alkaline. A recent study of the subject is that of E. Kelly and H. T. Parsons (22).

B. coli and a number of acid-fast bacteria, *B. smegmatis, B. timothy, B. Moelleri, B. adhaerens, B. subtilis, B. mycoides, Actinomyces,* and *Azobacterium,* can also produce thiamin, or so it seems from the data available (23). Sunderlin and Werkman also reported positive results with *Torula rosea* and *Oidium lactis. Monilia candida* was found to effect a vitamin B synthesis (24), as were *B. vulgatus* and *B. mesentericus* (25). *B. lactis aerogenes* and *Vibrio alcoligenes* (26) are other like cases. The principal difficulty with some of these studies is that no distinction was made between thiamin and the thermostable B factors by the rat assay methods used. However, qualitatively, if a positive effect on rat growth is obtained, the presence of some thiamin is indicated.

Use of Thiamin by Organisms

Higher plants. It may be reasonably inferred from what has been said about its occurrence in higher plants that thiamin is utilized by them physiologically. We must first summarize what has been learned regarding the function of thiamin in higher plants. For this purpose we shall refer to the earlier work of Robbins (27) and of P. R. White (28), who showed respectively that yeast extract was beneficial for the growth of isolated corn roots and tomato roots. Kögl and Haagen-Smit (29) studied the growth of pea embryos as affected by nutrilites, and in a very interesting paper disclose that the pure forms of biotin, thiamin, and oestrone (female hormone) all affect the growth favorably. Thiamin has more effect upon the growth of roots than on the shoots. Biotin and thiamin jointly have more effect than either separately. The thiamin and biotin contents of a single pea were estimated at 0.5γ and 0.1γ, respectively, which are approximately

the amounts required per excised embryo to produce maximum growth. The implication that storage in the seed is for the express purpose of providing for germination is unmistakable. Vitamin C was found without action.

Bonner (30) was the first to report similar effects on excised roots of the pea, using synthetic thiamin to the extent of 0.2 γ per c.c. of medium and with G. Axtman extended the work to other nutrilites (31). Vitamin C was found effective in increasing the growth of root and shoot, as was folliculin. Pantothenic acid was as effective on stem growth as thiamin, but less so as to root growth. Leaf development was much less marked than with intact embryos and was unaffected by any of these nutrilites. Root studies have a considerable advantage over embryos for such tests. With embryos there is considerable growth in the controls, so that the contrasts in the presence and absence of added nutrilites are less marked. The pea embryo carries in it enough of the nutrilites to be quite significant (32).

A like finding with respect to the action of pure thiamin on tomato roots was almost simultaneously reported by Robbins and Bartley (33), and further contributions have poured in from various sources during 1937. The partial efficacy of the thiazole portion alone in promoting tomato root growth and the complete adequacy of an equimolecular mixture of the thiazole and pyrimidine portions as a substitute for thiamin are among the interesting developments (34). The extent of the stimulation of these roots by mere traces of thiamin or its intermediates is astounding. Growth is proportional to added thiamin (or intermediates) over a wide range. (Fig. 19.) The above papers of Bonner and of Robbins have served to emphasize the idea first intimated by Kögl and Haagen-Smit that thiamin has to do peculiarly with root growth rather than with the tops of plants. The view that the roots depend on the upper parts of the plants for their supply seems well justified as a generalization.

Perhaps it will be well to mention at this point the observation that pigeons can be cured by very large amounts of the mixture of intermediates given by mouth. It seemed possible that synthesis of thiamin from the intermediates takes place in the digestive tract by the intervention of plant tissues undergoing the process of digestion, or by intestinal flora (35). However, recent experiments indicate that similar results are obtained by injection (36).

We may now consider the probable significance of thiamin in higher plants. The concentration of the substance in the seed has often been remarked, and it is of great interest to find that it has a special influence upon early root growth. At some other stage higher plants must synthesize the vitamin. This probably takes place in the tops. According to Hlavaty (37) the

Figure 19. Increase in growth of excised tomato roots with increasing amounts of thiamin chloride. The figures represent γ per 40 c.c. of medium. Courtesy of Drs. W. J. Robbins and Mary B. Schmidt.

thiamin content declines in germinating legume seeds, and synthesis does not begin till about a month later, and then only if the plants are in the sun. The B_1 content of cereal seeds also decreases during germination (38). It is also of interest to record the observation of Hunt *et al.* (39), that alfalfa, timothy, and clover hays cut when quite young are rich in thiamin and that the content declines with advancing age. The thiamin content seems to correspond, according to these authors, with the leafiness and greenness of the plant. This probably does not represent a transport of stored vitamin from the seed to the new-formed top, but indicates that the process of synthesis has already begun. The accumulation in the seed which must occur later during its ripening process presumably represents a migration from other tissues of the plant toward the seed, perhaps with a consequent impoverishment of the remainder of the plant. According to recent results of Bonner (private communication) it is already clear that synthesis of thiamin takes place in leaves and only in the light. Sugar-cane juice from the tops has been found richer in B_1 than juice from the butts of the stalks (page 234).

Does each higher plant actually synthesize its entire supply? It has been estimated that the thiamin content of grains grown on soils manured with artificial ferilizers is lower than that grown with barnyard manure (40). Cattle dung is reported to contain 0.1 γ per gram of dry weight (41). The fact that thiamin is so firmly adsorbed by clays makes it possible that the soil's store of the substance is conserved thereby against leaching. This may well have a bearing upon soil fertility, either by furnishing a supplementary supply of thiamin to higher plants directly or via soil bacteria. A very interesting development which may have an early practical importance is the use of thiamin in stimulating root formation in cuttings of plants (42).

Fungi. As we pass to the molds and fungi the need for thiamin is readily discerned. Probably the need is definitely associated with the saprophytic character of these plants. Yeast is ordinarily classed as a fungus, though it does not fit perfectly into the classification. Its case has already been sufficiently discussed so we shall next take up the higher fungi.

Some of the higher fungi can synthesize thiamin, *e.g.*, *Aspergillus oryzae* (43), *Aspergillus niger* (44), and other molds (45). Some other fungi are sensitive to a lack of thiamin. For the study of this class of organism we are largely indebted to the beautiful work of W. H. Schopfer. It is to be hoped that he will presently find opportunity to collect his results and an interpretation of them in a connected account. They appear at present in a very large number of papers, chiefly in *Archiv für Mikrobiologie, Zeitschrift für Vitaminforschung, Berichte der deutschen botanischen Gesellschaft, Comptes Rendus des Séances de l'Académie*

des Sciences, Paris, and in several other journals, largely during the years 1930 to 1937. They bear on many aspects of the subject, and it will not be possible to do them full justice here.

The following species have been shown to be unable to grow in the absence of either thiamin or its intermediates: *Phycomyces Blakesleeanus, Phycomyces nitens, Absidia ramosa, Mucor Ramannianus, Pilairia anomala, Parasitella simplex, Chaetocladium Brefeldi, Dicranophora fulva* (46). Others, while reacting appreciably to thiamin, are more sensitive to some other component of an extract of wheat germ, *e.g., Rhizopus suinus* and *R. Oryzeae.* Of all the organisms mentioned, *Phycomyces* has proved most suitable for thiamin testing. Others which were studied included *Absidia orchidis, Absidia glauca, Absidia repens, Mucor mucedo, Sporodinia grandis,* all of which react positively (47).

Microorganisms. The older literature as reviewed by Peskett (3) cannot be trusted very much as applying to the need of microorganisms for thiamin specifically. All experiments made prior to about 1930 and many since that time have involved extracts of natural substances which might well contain other significant substances besides thiamin. We will, therefore, refer the reader to Peskett's article rather than recount the experiments.

An exception will be made in the instance of *Streptothrix corallinus,* which Reader (48) found responded to additions of antineuritic vitamin concentrates with greatly improved growth. This work was done in the laboratory of R. A. Peters, who devoted years to the chemical isolation of thiamin. His preparations at this stage were undoubtedly approaching purity. A quantitative Streptothrix test for thiamin was proposed (49), but it was later found liable to give anomalous results in that antineuritic preparations rendered inactive for polyneuritic birds by heating in alkaline solutions were found still active for Streptothrix. R. A. Peters (50) offered the seemingly prophetic opinion that Streptothrix actually needs torulin (thiamin) and can reactivate alkalized torulin. In the light of later events, it seems probable that neutral or faintly alkaline solution splits the thiamin into its thiazole and pyrimidine components without destroying either portion. Strong or moderately strong alkali is likely to destroy the thiazole portion. As will be seen below, a mixture of these two intermediates or building stones of thiamin is, in general, equally as effective for the promotion of plant processes as is an equimolecular quantity of the two substances in the combined form. In some cases only one intermediate is required. For animals only the intact vitamin molecule is effective. In this distinction lies the fundamental weakness of plants and microorganisms as means of assay of foodstuffs for thiamin content. In so far as inactivation of thiamin occurs in the cooking of

food, it probably does so by reason of splitting the nuclei apart. Such a test of the cooked food may measure its activity for plants, not for animals. The use of other organisms such as Phytophthora may overcome this difficulty (see below).

As regards microorganisms in general, it seems that synthesis of thiamin takes place to such an extent that their need for the substance is not readily established. However, the general occurrence of thiamin in them is a cogent argument that they not only make thiamin but use it for their own purposes. A specific case in which proof of need for thiamin has been adduced is that of propionic acid bacteria (51). Naturally greater weight can be given to such recent studies as this because of the use of pure thiamin rather than crude extracts.

Another specific instance in which a microorganism is found to require an external supply of thiamin is found in the work of Knight (52). According to this work, which involved pure thiamin, *Staphylococcus aureus* requires thiamin (or its intermediates) and nicotinamide as well. One surmises that the fact that this organism grows as a parasite in the tissues of living animals may be responsible for its having requirements similar to that of the animal. It is, however, like many other plants in that the intermediates of thiamin suffice for it (see below).

Thiamin Intermediates

Schopfer, after testing the efficacy of various pure preparations of thiamin from natural sources (53), soon undertook the study of its derivatives. He found equal stimulation of Phycomyces by a mixture of the pyrimidine and thiazole products of thiamin as by thiamin itself, and offered the suggestion that the fungus utilizes thiamin by converting it into these constituent parts which constitute the true nutrilites (54). Similar experimental results with Phycomyces were obtained nearly simultaneously by others (55), who took the view that the organism probably utilized these intermediates to make thiamin. A similar study of tomato roots soon followed (34), which disclosed that while the thiazole alone increased growth measurably, the best result was obtained by equimolecular proportions of the two. For a discussion of the particular derivatives which were found to be of service the reader is referred to Chapters XV and XVI.

It has since been found that some other organisms, like the tomato root, are stimulated by the thiazole portion alone, some are stimulated by the pyrimidine portion alone, but many more are stimulated only when both portions are present. A few are stimulated by neither under the conditions tried. As an example, of eight species of Torulae (56) cultivated in a basal medium of mineral salts, asparagine and dextrose, two failed to grow either

in the original medium or in the presence of added thiazole, added pyrimidine, or when both intermediates were added (thiamin itself was not tried); two grew slowly in the medium but were unaffected by any of the supplements; two grew better in the presence of both intermediates but not in presence of either alone; the remaining two were stimulated by pyrimidine or by pyrimidine and thiazole but not by thiazole alone. By cultivating Phycomyces in the solutions in which Torula had grown, it was shown that in one case Torula had synthesized both thiazole and pyrimidine from the components of the basal medium. In another case in which Torula grew with the aid of the pyrimidine alone, synthesis of the thiazole had been effected. It is, therefore, evident that some Torulae can synthesize neither, some the pyrimidine and thiazole, some the thiazole alone. Some grow only moderately either with or without additions. Perhaps the medium is deficient for them in another respect. Some definitely appear to require something else for growth than the substance supplied by this medium, as they do not grow at all either with or without pyrimidine and thiazole additions. Perhaps the required substance is thiamin itself. Certain Phytophthora require the vitamin and are unable to use the intermediates satisfactorily (57).

These results again emphasize the probability that thiamin is a well-nigh universal growth factor. Its synthesis can be effected partially or completely by various organisms. But it seems to us highly probable that the vitamin itself is the end product which is utilized by plants. This is the view of Robbins, based chiefly on the finding that equimolecular proportions of the intermediates are best for Phycomyces. This is also true for pea roots (41). This view commends itself to us not only for the reasons outlined by Robbins but also because it appears far more consonant with the function of thiamin in the animal kingdom, where it is associated with carbohydrate metabolism as discussed in Chapter XXVI. Schopfer's thought (58) that it is the split products which function in plants seems less acceptable because of the demonstrated activity of thiamin pyrophosphate in the field of microorganisms (see page 344). It would seem strange indeed to the chemist that two such distinctive compounds should be needed as separate entities in plants and as a combination in animals. The presumption is certainly in favor of the view that thiamin itself is fundamental to carbohydrate metabolism in all life and that it represents a very primitive biochemical mechanism inherited from early stages of biological evolution.

Substitutes for Thiamin Intermediates

Very interesting studies have been undertaken as to the variations of the molecules of thiamin intermediates which are

permissible for utilization by plants (59). Nine pyrimidines have been studied as stimulants for pea root growth. They show that an amino group in position 6, as in thiamin, is essential. Similar study of twenty thiazoles has shown the necessity of an alcoholic side chain, which, however, may be in any one of a number of positions in the thiazole portion. None of the variants is significantly more active than the thiazole of thiamin for pea roots. For Phycomyces none of the variants of the thiazole is equally effective (60, 61). A more detailed disclosure of these findings as they bear on chemical structure is found in Chapters XV and XVI. The pyrimidines for Dr. Bonner's studies were supplied by Dr. A. R. Todd and by Dr. J. K. Cline; the thiazoles by Dr. E. R. Buchman. The hope has been that such studies would reveal what groups in the molecule are biologically functioning groups. In view, however, of Dr. Robbins' finding that the vitamin thiazole is so readily made *de novo* by many organisms, rigorous proof will be necessary that a reconstruction of these substitute intermediates to the pattern of the thiamin thiazole does not precede their utilization by plants. It may be, of course, that these substitute thiazoles go to form analogues of thiamin and that these are able to function in lieu of thiamin in enzyme systems (see Chapters XIV and XVI).

Thiamin and Insects

A number of the earlier studies of this subject suffer from confusion of the various B factors. Portier (62) proposed the idea that symbionts of insects may produce the necessary vitamins. Bacot and Harden (63) believed, as a result of experiments with the eggs of Drosophilae, sterilized with alcohol, that vitamin B is required by these organisms. Sweetman and Palmer (64) found the beetle Trilobium to require vitamin B. Koch (65) cultivated Anobium in sterile pea soup, but the larvae remained small and did not produce chrysalids unless yeast extracts were added. The sheep maggot fly, *Lucilia sericata,* requires three factors from yeast extracts for its growth. One of these appears to be the antineuritic vitamin, another one of the thermostable B factors (66). Flour beetles are said to require both thiamin and riboflavin (67).

An extended study of the requirements of *Drosophila melanogaster* by van't Hoog (68) is prefaced by a review of the previous literature. To secure the initial eggs he cultivated the flies on crushed ripe bananas in an agar gel which was periodically strewn with brewers' yeast as an addendum. The flies were induced to lay their eggs on strips of sterile filter paper which served to transfer the eggs to other vessels where they were sterilized for thirty-five minutes with alcohol and chloramine and then

washed with sterile water. The resulting sterile eggs were then hatched upon an agar gel in which were incorporated casein-peptone, autoclaved brewers' yeast, salt mixture, sugar, and the unsaponifiable fraction of peanut oil. Other portions of the agar gel had also incorporated in them various amounts of Jansen's crystalline thiamin ranging from 1/16 γ to ½ γ per 5 c.c. of gel. In the control tubes nearly all the eggs hatched into larvae, but the latter all died in about twelve days without producing a single chrysalid. With 1/16 γ thiamin, about 10 per cent of the larvae produced chrysalids, and of these one fifth tardily produced flies, which, however, were quite sterile. With ⅛ γ, 30 per cent of the larvae formed chrysalids, and nearly all of the latter passed into non-fertile flies. With ¼ γ, 60 per cent of normal chrysalids were produced, which after four days hatched fertile flies. With ½ γ, practically 100 per cent of the eggs produced chrysalids and, in turn, fertile flies. Thus thiamin influenced several different features, viz., the extent and rate of hatching of larvae from the eggs, the extent and rate of development of flies from larvae, and, finally, the fertility of the flies. Other vitamins were similarly studied. The flies apparently do not need vitamins A, D, or E. Riboflavin and some other ill-defined yeast factors were found to be required.

Summary

Thiamin plays a conspicuous role as a factor for the growth of plants and microorganisms, more so than any other vitamin.

An external supply of it promotes the vital processes even of organisms which seem to possess some capacity for its synthesis.

The universality of the function of thiamin in living cells is inferred partly from its very widespread occurrence and partly from evidence of the necessity of incorporating it in artificial culture media.

The principal site of synthesis seems to be in the tops (probably the leaves) of higher plants; lower plants and the roots of higher plants appear, in general, to require an external source of supply.

Thiamin is stored in seeds to provide for the needs of the plant during early growth.

A mixture of the intermediates of thiamin serves as a substitute for thiamin for many plants. This has permitted a study of the synthetic capacities of organisms for pyrimidine, for thiazole and for uniting the two nuclei. A wide variation has been encountered in the capacities of different organisms.

Insects also require thiamin.

BIBLIOGRAPHY

1. Williams, R. J.: Nutrilites. Science *67*, 607, 1928.
2. Kögl, F., and Tonnis, B.: Über das Bios-Problem. Darstellung von Krystallisiertem Biotin aus Eigelb. Zeit. physiol. Chem. *242*, 43, 1936.
3. Peskett, G. L.: Growth Factors of Lower Organisms. Biol. Reviews *8*, 1, 1933.
4. Bonner, J.: The Role of Vitamins in Plant Development. Botanical Review *3*, 616, 1937.
5. Williams, R. J.: The Vitamin Requirement of Yeast. A Simple Biological Test for Vitamin. Jour. Biol. Chem. *38*, 465, 1919.
6. Bachmann, F. M.: Vitamine Requirements of Certain Yeasts. Jour. Biol. Chem. *39*, 235, 1919.
7. Miller, W. L.: Wildier's Bios. Science *59*, 197, 1924.
8. Eastcott, E. V.: Wildier's Bios. The Isolation and Identification of "Bios I." Jour. Phys. Chem. *32*, 1094, 1928.
9. Williams, R. J., and Roehm, R. R.: Effect of Antineuritic Vitamin Preparations on the Growth of Yeast. Jour. Biol. Chem. *87*, 581, 1930.
10. Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D.: Pantothenic Acid, a Growth Determinant of Universal Biological Occurrence. Jour. Amer. Chem. Soc. *55*, 2912, 1933.
11. Williams, R. J., and Saunders, D. H.: Effect of Inositol, Crystalline Vitamin B_1 and "Pantothenic Acid" on the Growth of Different Strains of Yeast. Biochem. J. *28*, 1887, 1934.
12. Kögl, F., and Fries, N.: Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Zeit. physiol. Chem. *249*, 93, 1937.
13. Fulmer, E. I., Nelson, V. E., and Sherwood, F. F.: Nutritional Requirements of Yeast. I. The Role of Vitamines in the Growth of Yeast. Jour. Amer. Chem. Soc. *43*, 186, 1921.
14. Pavcek, P. I., Peterson, W. H., and Elvehjem, C. A.: Effect of Growth Conditions on Yield and Vitamin B_1 of Yeast. Ind. and Eng. Chem. *29*, 536, 1937.
15. Schultz, A., Atkin, L., and Frey, C. N.: A Fermentation Test for Vitamin B_1. Jour. Amer. Chem. Soc. *59*, 948, 1937.
16. Schopfer, W. H.: Vitamines et Facteurs de Croissance chez les Plantes. Recherches sur l'Action de Divers Extraits Vegetaux sur le Developpement de *Phycomyces*. Arch. Mikrobiol. *7*, 156, 1936.
17. Schopfer, W. H.: Les Vitamines Cristallisées B comme Hormones de Croissance chez un Microorganisme (*Phycomyces*). Arch. Mikrobiol. *5*, 511, 1934.
18. Schopfer, W. H.: Recherches sur l'Action des Produits Animaux sur le Developpement d'un Microorganisme (*Phycomyces*). Arch. Mikrobiol. *8*, 231, 1937.

19. Bechdel, S. I., Honeywell, H. E., Dutcher, R. A., and Knutsen, M. H.: Synthesis of Vitamin B in the Rumen of the Cow. Jour. Biol. Chem. *80*, 231, 1928.
20. Guerrant, N. B., Dutcher, R. A., and Tomey, L. F.: Effect of the Type of Carbohydrate on the Synthesis of the B Vitamins in the Digestive Tract of the Rat. Jour. Biol. Chem. *110*, 233, 1935.
21. Griffiths, W. H.: B and G Avitaminosis in Cecectomized Rats. Jour. Nutrition *10*, 667, 1935.
22. Kelly, E., and Parsons, H. T.: Some Quantitative Studies of Refection in the Rat. Jour. Nutrition *13*, 453, 1937.
23. Sunderlin, F., and Werkman, C. H.: Synthesis of Vitamin B by Microorganisms. Jour. Bact. *16*, 17, 1928.
24. Hoet, J., Leclef, G., and Delrue, G.: Synthesis of Vitamin B by Microorganisms. Arch. Intern. Physiol. *19*, 129, 1924.
25. Schieblich, M.: Vergleichende Untersuchungen über die Fahigkeit de Vitamin-B-Bildung durch Bac. vulgatus (Flügge) Migula und Bac. Mesentericus (Flügge) Lehm et Neum. Biochem. Zeit. *220*, 394, 1930.
26. Schieblich, M.: Über den Einfluss der Zusummensetzung des Nährbodens auf die Vitamin-B-Bildung durch Bakterien. Biochem. Zeit. *259*, 19, 1933.
27. Robbins, W. J.: Cultivation of Excised Root Tips and Stem Tips under Sterile Conditions. Bot. Gaz. *74*, 59, 1922.
28. White, P. R.: Potentially Unlimited Growth of Excised Tomato Root Tips in a Liquid Medium. Plant Physiol. *9*, 585, 1934.
29. Kögl, F., and Haagen-Smit, A. J.: Biotin und Aneurin als Phytohormone. Ein Beitrag zur Physiologie der Keimung. Zeit. physiol. Chem. *243*, 209, 1936.
30. Bonner, J.: Vitamin B_1, a Growth Factor for Higher Plants. Science *85*, 184, 1937.
31. Bonner, J., and Axtman, G.: Growth of Plant Embryos *in Vitro*. Preliminary Experiments on the Role of Accessory Substances. Proc. Nat. Acad. Sci. *23*, 453, 1937.
32. Bonner, J., and Addicott, F.: The Cultivation *in Vitro* of Excised Pea Roots. Bot. Gaz. *99*, 144, 1937.
33. Robbins, W. J., and Bartley, M. A.: Vitamin B_1, and the Growth of Excised Tomato Roots. Science *85*, 246, 1937.
34. Robbins, W. J., and Bartley, M. A.: Thiazole and the Growth of Excised Tomato Roots. Proc. Nat. Acad. Sci. *23*, 385, 1937.
35. Robbins, W. J., Bartley, M. A., Hogan, A. G., and Richardson, L. R.: Pyrimidine and Thiazole Intermediates as Substitutes for Vitamin B_1. Proc. Nat. Acad. Sci. *23*, 388, 1937.
36. Robbins, W. J.: Private communication.
37. Hlavaty, J.: Sur la Disparition de la Vitamine B des Graines Germant dans un Germoir ou dans le Sol. Compt. Rend. Soc. Biol. *100*, 587, 1929.

38. Kuthy, A. von: Changes in the Content of Vitamin B_1 and Vitamin C in Germinating Cereal Grains. Proc. 5th Int. Cong. Tech. Chem. Agric. Ind., Holland *1,* 119, 1937.
39. Hunt, C. H., Record, P. R., and Bethke, R. M.: Influence of the Stage of Maturity on the Chemical Composition and the Vitamin B (B_1) and G Content of Hays and Pasture Grasses. Ohio Agric. Exp. Sta. Bull. 576, 1936. See also Jour. Agri. Res. *51,* 251, 1935.
40. Rowlands, M. J., and Wilkinson, B.: Vitamin B Content of Grass Seeds in Relationship to Manures. Biochem. J. *24,* 199, 1930.
41. Bonner, J.: Private communication.
42. Went, F. W., Bonner, J., and Warner, G. C.: Aneurin and the Rooting of Cuttings. Science *87,* 170, 1938.
43. Scheunert, A., and Schieblich, M.: Über Vitaminbildung durch *Aspergillus oryzae.* Biochem. Zeit. *286,* 66, 1936.
44. Fawns, H. T., and Jung, A: The Mineral Content of the Skin of Rats Suffering from Beriberi with Investigations on the Formation of Vitamin B_1 by Molds. Biochem. J. *27,* 918, 1933.
45. Gorcica, H. J., Peterson, W. H., and Steenbock, H.: Nutritive Value of Fungi. II. The Vitamin B_1, G and B_4 Content of the Mycelium of *Aspergillus sydowi.* J. Nutrition *9,* 691, 1935.
46. Schopfer, W. H.: Un Test Vegetal pour la Vitamine B_1. Zeit Vitaminforsch. *4,* 67, 1935.
47. Schopfer, W. H.: Sur la Synthese d'un Facteur de Croissance par un Microorganisme. Compt. Rend. Acad. Sci. Paris *199,* 1656, 1934.
48. Reader, V.: Effect of the Addition of Growth Promoting Substances to the Synthetic Medium on the Growth of *Streptothrix corallinus.* Biochem. J. *22,* 434, 1928.
49. Orr-Ewing, J., and Reader, V.: *Streptothrix corallinus* in the Estimation of Vitamin B_1. Biochem. J. *22,* 440, 1928.
50. Peters, R. A.: Harben Lectures II. Jour. State Med. *38,* 63, 1930.
51. Tatum, E., Woods, H., and Peters, W.: Growth Factors for Bacteria. V. Vitamin B_1, a Growth Stimulant for Propionic Bacteria. Biochem. J. *30,* 1898, 1936.
52. Knight, B. C. J. G.: The Nutrition of *Staphylococcus aureus.* The Activities of Nicotinamide, Aneurin (Vitamin B_1) and Related Compounds. Biochem. J. *31,* 966, 1937.
53. Schopfer, W. H., and Jung, A.: Recherches sur l'Emploi d'un Test Végétal pour l'Etude de la Vitamine B_1. Essai de Standardisation. Compt. Rend. Assoc. libre Physiol. Suisse, June 1935.
54. Schopfer, W. H., and Jung, A.: Physiologie Vegetale. L'Action des Produits de Désintégration de l'Aneurine sur Phycomyces. Le Second Facteur de Croissance des Mucorinées. Compt. Rend. Acad. Sci. Paris *204,* 1500, 1937.
55. Robbins, W. J., and Kavanagh, F.: Intermediates of Vitamin B_1 and Growth of Phycomyces. Proc. Nat. Acad. Sci. *23,* 499, 1937.
56. Robbins, W. J., and Kavanagh, F.: Intermediates of Vitamin B_1 and the growth of *Torula.* Plant. Physiol. (In press.)

57. Robbins, W. J.: Organisms Requiring Vitamin B_1. Proc. Nat. Acad. Sci. *24*, 53, 1938.
58. Schopfer, W. H.: L'Action des Constituants de l'Aneurine sur les Levures (*Rhodotorula rubra et flava*). Compt. Rend. Acad. Sci. Paris *205*, 445, 1937.
59. Bonner, J.: (In press.)
60. Robbins, W. J., and Kavanagh, F.: Specificity of Pyrimidine for *Phycomyces Blakesleeanus*. Proc. Nat. Acad. Sci. *24*, 141, 1938.
61. Robbins, W. J., and Kavanagh, F.: Specificity of Thiazole for *Phycomyces Blakesleeanus*. Proc. Nat. Acad. Sci. *24*, 145, 1938.
62. Portier, P.: Recherches sur les Organismes Symbiotiques dans la Serie Animale. Compt. Rend. Acad. Sci. Paris *165*, 196, 1917.
63. Bacot, A. W., and Harden, A.: Vitamin Requirements of *Drosophila*. I. Vitamins B and C. Biochem. J. *16*, 148, 1922.
64. Sweetman, M. D., and Palmer, L. S.: Insects as Test Animals in Vitamin Research. I. Vitamin Requirements of the Flour Beetle, *Tribolium confusum* Duval. Jour. Biol. Chem. *77*, 33, 1928.
65. Koch, A.: Über das Vorkommen von Mitochondrien in Mycetocyten. Biol. Zentbl. *3–4*, 109, 1933.
66. Hobson, R. P.: Growth of Blowfly Larvae on Blood and Serum. I. Response of Aseptic Larvae to Vitamin B. Biochem. J. *27*, 1899, 1933.
67. Street, H. R., and Palmer, L. S.: Requirement of the Flour Beetle for Vitamins in the B Group. Proc. Soc. Exp. Biol. Med. *32*, 1500, 1935.
68. Van't Hoog, E. G.: Aseptic Culture of Insects in Vitamin Research. Zeitschr. f. Vitaminforsch. *4*, 300, 1935.

CHAPTER XXIX

RECAPITULATION

In the foregoing pages we have endeavored to outline the observations about thiamin which have withstood critical analysis. We have seen that our first knowledge of it originated in the existence of an Oriental disease, characterized by a diversity of disturbances both physiological and anatomical. For reasons which we do not yet fully understand, the lesions and clinical signs which are most conspicuous concern the nervous system, and accordingly we speak without hesitation of beriberi as polyneuritis.

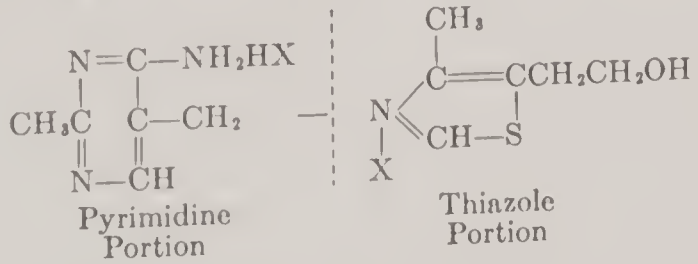
Accumulating evidence, however, makes it clear that we have been prone in the past to attach too narrow a significance to the role of the substance in physiological processes and to take too limited a view of the prevalence of the deficiency in human diets. The consequences of thiamin deficiency upon the operation of nerve function cannot now be regarded as due to wholly unique needs of nervous tissue. All tissues alike need thiamin for the performance of their normal functions, and the conspicuous character of the nervous derangements of beriberi must be attributed, at least tentatively, to the fact that the tissues of the nervous system contain the most highly organized and delicately balanced of all living cells. An impulse moving along a nerve fiber is a disturbance of an electrical nature which can be measured and followed with physical instruments and can be plotted in graphic and precise form. From such studies we know that, to a peculiar degree, the nerve fiber which has just functioned is no longer the identical physicochemical system that existed previously. After the passage of a train of impulses, its sensitivity has diminished. Brief but measurable intervals of time must elapse before the physiological processes in the cells of the fiber can restore it to the state which it previously exhibited. In use, the nerve fiber represents an extremely delicate balance between the processes of decay or of wear and the processes of repair. Furthermore the energy generated by the nerve fiber's chemical processes is essential to its function as nerve. It can be rendered inert and dormant almost immediately by curtailment of its oxygen supply, can be kept in that state for a substantial part of an hour, and restored again to activity by renewal of its respiratory function.

The nervous manifestations of beriberi are therefore most plausibly to be considered as merely the most extreme and conspicuous evidences of a lack which is equally felt in all tissues.

Likewise the prevailing geographical limitation of beriberi to the Orient is due to accident of food habits. It is merely a conspicuous case of a deficiency which in milder degree occurs in all parts of the globe and in large numbers of people representing the less well fed, the physiologically less efficiently organized, or the victims of unusual strains upon physical resources; for example, the burdens of hard labor, of motherhood, or of disabilities due to infectious disease. No one can say categorically how largely this is true or what proportion of humanity is affected, but such a view of vitamin B_1 deficiency is basically sound in principle.

Such a view rests not only upon evidence of subclinical signs of thiamin deficiency in large numbers of patients, not only upon the extent and character of the therapeutic responses which are often evoked by administration of the substance, nor even primarily upon the evidence that the substance though very widespread in foodstuffs is not superabundant in any and is scanty in most. It rests far more upon the finding that the substance appears to play a role in the metabolism of every living cell, whether plant or animal, whether unicellular organism or one of the units of those very complex assemblies of organized matter which constitute the higher forms of life. Again we cannot be categorical, for not all cells have been examined. However, an extremely wide diversity of tissues have been studied. Everywhere we find evidence either of the presence of the substance, apparently as a consequence of living process, or of a need for an external source of supply of it in order that life may continue. In many organisms both the presence of the substance in normal tissue and the need of it for normal function have been amply demonstrated.

Chemically thiamin, or vitamin B_1, has a number of rather unique features. It contains two nuclei, the pyrimidine and thiazole, bound together in a not very firm union through a methylene bridge in position 5 of the former extending to the nitrogen of the latter by quadricovalence.



Pyrimidine Portion — Thiazole Portion

It is probably significant that the stability of the internuclear bond appears to be at or near a minimum in the neutral range which tends to prevail in living tissues.

Some plant tissues are able to profit by an external supply of the thiazole portion of the vitamin alone, probably because the tissues contain or can synthesize readily the pyrimidine portion; others exhibit a more conspicuous benefit from an external supply of the pyrimidine portion. Some are able to synthesize both portions. In certain instances synthesis appears to supply both abundantly and most (but not all) plant tissues have an ample capacity to unite the two portions to make the vitamin. However, in the majority of organisms the synthetic capacity is limited at one stage or another of the series of chemical reactions, or in one stage of the life cycle, or in some part of the organism to such an extent that an external additional supply is capable of promoting measurably the vigor of the life processes. *This is very widely true.*

In all animals and in at least some saprophytic plants the power for complete synthesis of the vitamin is almost entirely absent. We thus gain the impression that the rate of synthesis is, in general, marginal so that Nature has a struggle to make and maintain adequate supplies of the vitamin to keep life moving forward at an optimal pace. She must resort to transport of supplies of the substance from actively growing parts to other less-favored parts. She must resort to storage during the growing season for use at the end of a period of dormancy as in the germinating seed. She must utilize the residues left in dying tissues of one genus for the propagation at one stage or another of other genera. In short, she carries on a hand-to-mouth economy of the substance, utilizing the surplus of some of her children to supply the necessities of others. Probably the instability of the substance under the conditions prevailing in tissues contributes to the meagerness of the earth's store.

It is small wonder that man, like other products of the evolutionary process, should share betimes in the shortage. The artificiality of his habits, the varied character of his habitats, very possibly the tempo of his life, make him peculiarly susceptible. Man, like other animals, needs the vitamin for the metabolism of the non-fat portion of his food. It is a striking fact that for all species of animals the requirement is nearly the same to the best of our present knowledge and in each case it bears a close relationship to the amount of carbohydrate (and protein residues) metabolized.

The vitamin takes part in the carbohydrate metabolism of plants as well as of animals, probably by virtue of the same essential mechanisms. In association with appropriate specific proteins, it constitutes, in the form of its pyrophosphate, the prosthetic group of several enzyme systems which are needed at one or more stages of the metabolism of carbohydrates. Accordingly it seems likely that the vitamin B_1 requirement of all

living things will turn out to be essentially alike in terms of molecules of the metabolite which are converted into energy. The relationship of thiamin to other bioagencies of carbohydrate metabolism, *e.g.,* insulin, deserves a great deal of further study.

It would be misleading to omit to say that thiamin is probably not the only substance which plays a more or less universal and perhaps kindred role in living cells. Riboflavin (vitamin B_2) and vitamin C both seem to have some such properties. Nicotinic acid or its amide may also be a rival for distinctive honors. However, vitamin B_1 is outstanding with respect to the apparent universality of its function in living cells and the degree of dependence of the cells upon an adequate supply of it. The lack of no other accessory substance leads to so early, so profound, and so universal a disaster, according to our present evidence.

The self-respecting physician need therefore have no fear that he is being beguiled by the blandishments of a cure-all, if he is tempted to extend his clinical experimentation with thiamin far afield beyond the disorders which are intimately and obviously related to beriberi. He will, however, do wisely to exercise the greatest skepticism regarding how much should be attributed to thiamin and how much to other lesser known causes. The confusing and contradictory state of the present literature is largely due to the fact that the thing we once supposed to be a single substance, which we called vitamin B, has turned out to consist of a dozen or so separate entities, each contributing to some degree to the total effect of crude products, such as yeast or liver extract. It would be idle to suppose that we have safely passed all these pitfalls.

On the other hand, it will be most surprising if we do not within the next decade find a great province for exploration in the field of the inter-relationships among the vitamins and between the vitamins and the hormones. Many enzymic systems are now known to be linked in definite sequences of chemical reactions, and further such links are doubtless to be found. Probably we shall learn the explanation of many obscure points regarding the character and etiology of the constitutional diseases if we look upon our present knowledge of thiamin not as a finished voyage but as a point of departure for new discoveries.

In the meantime thiamin has a definite and relatively well proven field of usefulness in the day-to-day practice of medicine. From clinical studies also it is becoming clear that vitamin B_1 is essential for the life of the cells. If not present in suitable quantities, the cells suffer and disease follows. The authors and others note that vitamin B_1 deficiency is common and that it can be corrected by the physician if therapy is directed along three lines: (*a*) elimination of conditions causing excess require-

ment for vitamin B_1, (*b*) the administration of the substance in adequate amounts to correct the deficiency, (*c*) the treatment of coexisting diseases. In the preceding chapters the authors have discussed the clinical indications for the use of thiamin hydrochloride (vitamin B_1) in a large number of clinical entities associated with undernutrition and peripheral neuritis, and have also stressed the need for the physician to administer this substance in order to prevent the development of subclinical deficiency.

INDEX OF NAMES

Aalsmeer, W. C., 65, 75.
Abderhalden, E., 74, 101, 105, 244, 254, 275, 276, 279, 320, 321, 326, 329, 347.
Abdou, N. T., 33.
Addicott, F., 389.
Adler, S., 156.
Aeschlimann, J. A., 171.
Albeck-Aarhus, 52.
Albert, J., 32.
Allen, A. W., 27, 31.
Alvarez, W. C., 69, 76, 367.
Ammerman, M., 169, 204, 208, 209, 215, 222, 223, 232, 253, 280, 284, 285, 287, 297, 301, 304, 308, 311, 312.
Andersag, H., 154, 155, 156, 179, 189, 196.
Anderson, J. E., 298.
Andrews, V. L., 32, 63, 71, 75, 77.
Angle, F. E., 47, 55.
Aoki, K., 28.
Arakawa, T., 72, 77, 369.
Aring, C., 11, 15, 45, 49, 53, 55, 74, 81, 83.
Ariyama, T., 370.
Armour, J. C., 358, 366.
Arnold, A., 202, 221, 284, 297.
Asakura, K., 72, 77, 326, 330, 369.
Ashford, B. K., 45, 53.
Atkin, L., 220, 225, 387.
Auhagen, E., 344, 354.
Axtman, G., 380, 388.
Aykroyd, W. R., 33, 104, 106, 120, 230, 234.

Bachmann, F. M., 220, 225, 387.
Bacot, A. W., 385, 390.
Baglioni, A., 361, 368.
Baker, A. Z., 105, 109, 119, 160, 170, 212, 224, 232, 241.
Baker, B. M. Jr., 54.
Baker, L. C., 368.
Balkin, E. R., 298.
Barelare, B., 298.
Barger, G., 161, 170, 174, 179, 342.
Barr, D. P., 67, 76.
Barry, M. M., 76.
Bartley, M. A., 189, 380, 388.
Beard, H. H., 308.
Bechdel, S. J., 388.
Beckh, W., 55.
Bell, M. E., 327, 330.
Benedict, W. L., 92.
Bennholdt-Thomsen, C., 14.
Bentley, A. J. M., 30, 62, 75.
Bergel, A., 54.
Bergel, F., 154, 156, 161, 170, 171, 179, 190, 196.
Berkwitz, N. J., 41, 51.
Bernard, 47.
Bernhardt, K. S., 292, 298.
Bernstein, R. E., 253, 255.
Best, C. H., 355.
Bethell, F. H., 54.
Bethke, R. M., 242, 389.
Bickel, G., 92.
Bingham, A. W., 43, 52.
Birch, T. W., 211, 223, 224, 367.
Bird, H. R., 137.
Birge, W. S., 32.
Bishop, K. S., 60, 75.
Blackfan, K. D., 13.
Blankenhorn, M. A., 36, 38, 51, 136.
Block, R. J., 207, 223.
Bloombergh, H. D., 57, 73.
Bloomfield, A. L., 51.
Bonner, J., 192, 193, 195, 373, 380, 381, 385, 387–390.
Bontius, J., 17, 27.
Bordley, J., 54.
Boudreau, F. G., 92.
Boyd, W. C., 265, 298.
Braddon, W. L., 28, 120, 257, 264.
Briganti, A., 326, 330.
Broccard, R., 15, 39, 48, 51.
Brodie, J. B., 249, 255.
Brosteaux, J., 340, 353.
Brown, C. J., 32, 47, 55.
Brown, R. A., 274.
Browning, E., 60, 74.
Bruck, A., 370.
Bruckman, F. S., 26, 33.
Buchanan, K. S., 79, 83, 262, 265, 274, 279, 367, 368.
Buchman, E. R., 150, 157, 170, 179, 189, 193, 195, 196, 385.
Bullmore, C., 32.
Burn, J. H., 215, 221, 224.
Burr, G. O., 293, 294, 298.
Burton, G. W., 159, 170.

Cannon, A., 30, 265.
Cantineau, G., 33.
Caridroit, F., 347.
Carmichael, E. A., 54.
Carter, C. W., 136, 137, 211, 222–224.
Caspari, W., 29.
Castle, W. B., 53, 54, 78.
Cerecedo, L. R., 143, 190, 193, 195, 222, 225, 312, 315, 326, 330.
Chamberlain, W. P., 29, 57, 73, 139, 142.
Chase, E. F., 202, 282, 284, 285, 312.
Chen, K. K., 157.
Chiba, M., 72, 77, 363, 369.
Chick, H., 159, 170, 202, 216, 222.
Chinn, A. B., 53, 58, 73.
Choroschko, W., 31.
Christian, W., 162, 170.
Church, A. E., 370.
Church, C. F., 213, 224, 273, 278, 346, 355.
Clark, E., 58, 74.
Clark, L. M., 171.
Clarke, H. T., 147, 157, 171, 193, 195.
Clay, J., 298, 357, 366.
Cline, J. K., 27, 51, 87, 93, 155–157, 171, 173, 175, 189, 217, 225, 385.
Cobb, S., 9, 14, 35, 50, 55, 120.

Cody, C. C., 92.
Coggeshall, H. C., 9, 14.
Colbert, C. M., 29, 37, 39, 51.
Colby, M. G., 298.
Collazo, J. A., 276, 279, 318, 319, 329.
Collins, E. N., 76, 358, 366.
Conos, B., 31.
Console, J., 361, 368.
Cook, B. B., 137.
Cooley, T. B., 298.
Cooper, C., 136.
Cooper, E. A., 228, 241, 244, 250, 254, 256, 257, 264.
Copping, A. M., 231, 232, 235, 241.
Corkill, B., 327, 330.
Couréménos, 31.
Courtois, A., 52.
Courville, C., 55.
Coward, K. H., 208, 215, 221, 224.
Cowgill, G. R., 14, 32, 37, 51, 59, 69, 71, 74, 76, 94, 95, 100, 101, 105, 108, 120, 207, 216, 223, 227, 230, 234, 241, 261, 265, 284, 288, 297, 300–315, 357, 361, 366, 368.
Cracas, L. J., 222.
Crowell, B. C., 29, 306, 308.
Cunningham, R. N., 14.

Dalldorf, G., 60, 74, 120, 358, 366.
Daniel, E. P., 242.
Das, N. B., 355.
Davis, M., 127, 129.
Davison, C., 57, 73.
Davison, H. G., 137.
de Calvi, M., 47.
De Jong, S., 340, 353.
Delrue, G., 388.
Dennett, R. H., 76.
Derks, T. J. G., 136.
D'Esopo, L. M., 26, 33.
Desrochers, G., 40, 51.
Deuel, H. J. Jr., 76.
De Witt, J., 367.
De Wolf, H. F., 36, 51.
Di Mattei, P., 201, 221, 362, 369.
Dobringer, K., 55.
Donath, W. F., 17, 27, 139, 140, 143, 146, 200, 201, 206, 213, 221, 241, 285, 312, 315.
Doyle, M. E., 224.
Drummond, J. C., 60, 74, 109, 119, 160, 170, 223, 232, 241, 272, 276, 278, 279, 337, 339, 347, 352, 368.
Drury, A. N., 211, 223, 224.
Dubois, E. V., 265.
Dupouy, R., 52.
Dutcher, R. A., 272, 274, 278, 337, 352, 388.
Duvernay, 31.

Easteott, E. V., 387.
Eckler, C. R., 157.
Eddy, W. H., 60, 74, 120, 366.
Edie, C. S., 126, 142.
Egan, P. B., 262, 265.
Eggleton, P., 321, 323, 329.
Eijkman, C., 28, 57, 73, 124, 125, 128, 130, 136, 138, 139, 159, 170, 197, 200.
Elias, H. L., 298.
Ellinger, P., 55.
Ellis, W. G., 28, 62, 75.
Elsom, K. O., 14, 30, 76.
Elvehjem, C. A., 133, 136, 137, 160, 170, 178, 202, 221, 222, 228, 235, 243, 284, 297, 340–342, 353, 354, 387.
Elvove, E., 58, 73.
Ely, F. A., 52.
Embden, 335, 363.
Emmett, A. D., 135.
Enright, J. I., 33.
Eubanas, F., 17, 27.
Euler, H. v., 137, 162, 179.
Eusterman, G. B., 31, 54.
Evans, H. M., 75, 77, 81, 267–279, 293, 294, 299.
Evans, J., 60, 74, 83.
Evans, W. H., 142.

Fabre, R., 362, 369.
Fairley, N. H., 53, 78.
Falta, W., 33.
Farnum, M. B., 358, 366.
Fawns, H. T., 362, 369, 389.
Feil, H., 59, 66, 74.
Fellinger, K., 15.
Field, H., 76.
Findlay, G. M., 341, 353, 368.
Finkelstein, J., 156, 189.
Fischer, H., 55.
Fisher, R. B., 353.
Fitts, J. B., 69, 76.
Fletcher, W., 28, 125, 128.
Foley, M. A., 69, 76, 367.
Ford, R. K., 52.
Forman, G. W., 43, 52.
Fouts, P. J., 43, 46, 52, 54, 136.
Fraenkel-Conrat, H. L., 179, 190, 196.
Fraga, C., 29.
Frandsen, H., 14.
Fraser, H., 28, 109, 120, 125, 129, 138, 142, 295, 305, 308.
Freudenberg, R., 92.
Freudenberg, W., 312, 315.
Freudenthal, P., 222.
Frey, C. N., 220, 225, 387.
Fridericia, L. S., 222.
Fries, N., 387.
Frisch, R. A., 59, 74.
Fritzsche, H., 179.
Frölich, T., 127.
Frost, D. V., 137.
Fujisaki, K., 28.
Fulmer, E. I., 234, 242, 387.
Funk, C., 30, 120, 124, 126, 129, 133, 138, 130, 142, 197, 258, 264, 266, 276, 277, 279, 318, 329.

Gabriel, 190.
Gaddie, R., 370.
Gaehtgens, G., 43, 53.
Galamini, A., 355, 360, 367.
Gallowitsch, P., 15.
Garrod, A. E., 55.
Gavrilescu, N., 352.
Geiger, A., 71, 77, 364, 370.
Gilman, A. Z., 59, 69, 74.
Gin, S. Y., 30.
Gleim, W., 137.
Goldberger, J., 131, 136.
Goldhamer, S. M., 54.
Goodall, A., 78.
Goodhart, R., 21, 32, 38, 40, 51, 102, 105.
Goodman, J. G., 269, 274, 278, 360, 367.
Goodyear, G. H., 387.
Gorcica, H. J., 389.
Goudsmit, J., 217, 225.
Graham, A., 30, 298.
Graham, C. E., 357, 366.
Grant, J. M., 119.
Green, D. E., 340, 353.
Greene, J. A., 46, 54.
Greenfield, J. G., 54.
Gregory, E., 272, 278.
Grewe, R., 147, 154–157, 190, 196.
Griffith, J. P. C., 13.

Griffith, W. H., 248, 254, 357, 366, 379, 388.
Grijns, G., 28, 125, 138, 305, 308.
Grose, M. R., 159, 170.
Gross, L., 321, 323, 329.
Gudjonnson, S., 222.
Guerrant, N. B., 272, 273, 278, 279, 388.
Guerrero, M. S., 32.
Guha, B. C., 160, 170, 269, 276, 278, 341, 353.
Günther, H., 55.
Gurin, S., 157, 171, 195.
Gustafson, G. W., 43, 52.
György, P., 136, 137.

Haagen-Smit, A. J., 379, 388.
Haas, S. V., 31
Habenkamp, T., 92.
Haden, R. L., 5, 13.
Hall, F. C., 92.
Halliday, N., 170.
Hamil, B. M., 298.
Hanes, F. M., 13, 53.
Haplin, J. G., 137.
Harden, A., 385, 390.
Hardy, M. C., 292, 298.
Hargreaves, F. J., 298, 357, 366.
Harrelson, R. T., 69, 76, 367.
Harris, I., 308.
Harris, L. J., 120, 209, 211, 215, 223, 224, 232, 241, 250, 255, 298, 357, 366.
Hart, E. B., 137, 160, 170.
Hartley, J. G., 235, 242.
Hartsock, C. L., 13, 31.
Hartung, E. F., 76.
Hashimoto, H., 18, 28.
Hasselbach, F., 55.
Hayasaka, E., 353.
Hayman, D. F., 156.
Hecht, G., 79–83.
Hehir, P., 29.
Heiman, M., 15, 50, 56.
Heller, V. G., 234, 242.
Helm, J. B., 32.
Helmer, O. M., 80, 83, 136, 250, 255.
Hendrick, E. G., 130, 131, 136, 361, 368.
Hennessy, D. J., 143, 222, 225, 313, 315, 326, 330.
Hermano, A. J., 17, 27.
Herrmann, G. R., 66, 75.
Hertz, S., 30.
Hess, W. R., 244, 254.
Hesse, E., 15.
Heyroth, F. F., 171.
Higginbotham, U., 66, 75.
Hills, G. M., 343, 354.
Hirano, Y., 362, 369.
Hiraoka, T., 362, 369.
Hirota, Z., 17, 27.
Hlavaty, J., 380, 388.
Hoagland, R., 228, 241.
Hobson, R. P., 390.
Hoefer, C., 291, 298.
Hoesslin, R., 52.
Hoet, J., 388.
Hofer, J. W., 178, 345, 354.
Hofmann, J., 41, 52.
Hofmeister, E., 143.
Hofer von Lobenstein, M., 14,
Hogan, A. G., 137, 189, 276, 279, 290, 298, 388.
Holaday, D., 387.
Holiday, E. R., 179.
Holmes, B., 33.
Holmes, B. E., 338, 352.
Holmes, E. G., 338, 352.
Holst, A., 127, 159, 170.
Holt, L. E., 298.
Honeywell, H. E., 388.
Hoobler, B. R., 13.
Hoogenhuijze, C. J., 136.
Hopkins, F. G., 126, 127, 129.
Huang, K. K., 30, 63, 75.
Hume, E. M., 159, 170.
Hunscher, H. A., 298.
Hunt, C. H., 242, 381, 389.
Huntsman, M. E., 355.
Hunter, D., 78.

Igarvidez, P. G., 29.
Imai, T., 156, 174, 179.
Inaizumi, M., 367.
Inawashiro, R., 353.
Isaacs, R., 54.
Ito, K., 28.

Jackson, J., 34, 50.
Jacob, A., 179, 190, 196.
James, G. V., 78.
Janowskaja, B., 269, 278.
Jansen, B. C. P., 17, 27, 109, 119, 129, 130, 135–137, 139, 140, 143, 146, 200, 201, 206, 207, 209, 213, 215, 217, 221, 223, 225, 254, 258, 264, 285, 312, 315, 364, 370, 386.
Jarussowa, N. S., 249, 255.
Jeans, P. C., 14.
Jeghers, H., 14.
Joachimovitz, P., 71, 77.
Joffe, P. M., 29, 37, 51, 358, 366.
Johansen, G., 222.
Johnson, P. E., 137.
Johnson, R. E., 341, 353.
Johnson, T. B., 190.
Johnson, W. A., 347, 348, 355.
Jolliffe, N., 14, 21, 29, 32, 37, 39, 40, 51, 102, 105, 358, 366.
Jones, B., 242.
Jones, C. M., 27, 31, 67.
Jones, W. A., 76.
Jordan, W. R., 48, 55.
Jukes, T. H., 3, 136, 137.
Jung, A., 180, 189, 220, 225, 362, 369, 389.

Kagawa, S., 29.
Kagoshima, S., 28.
Kakinuma, G., 28.
Kamachi, T., 367.
Kanao, R., 28.
Karimullah, 179, 190.
Karr, W. G., 59, 74.
Karrer, P., 132, 170, 179.
Karrer, W., 217, 225.
Kaszuba, F. J., 143, 222, 225, 313, 315.
Kauffman-Cosla, O., 361, 368.
Kavanagh, F., 180, 181, 189, 195, 389.
Kawaguchi, Z., 306–308.
Kawakita, S., 28.
Kawashima, K., 28.
Keefer, C. S., 13, 31, 59, 63, 65, 67, 74.
Keenan, J. A., 160, 170.
Kellogg, M., 60, 74, 358, 366.
Kelly, E., 137, 274, 279, 379, 388.
Kemmerer, A. R., 247, 254, 272, 278.
Kempf, G. F., 46, 54.
Kepler, E. J., 27, 30.
Keresztesy, J. C., 17, 27, 51, 136, 143, 156, 170, 189, 196, 213, 223, 224, 259.
Khlestkina, Z. N., 241.
Kik, M. C., 298, 355, 367, 370.
Kilbourne, E. D., 57, 73.
Kinnersley, H. W., 71, 77, 139, 141, 143, 156, 171, 208, 215, 222–224, 338, 341, 344, 352–354.

Kircher, A., 165, 171.
Kline, O. L., 137, 160, 170.
Klippel, M., 31.
Klotz, B. H., 223, 308.
Knack, A. V., 33.
Knapp, E., 14.
Knight, B. C. J. G., 179, 181, 189, 195, 383, 389.
Knott, E. M., 71, 77, 253, 255.
Knutsen, M. H., 388.
Koch, A., 385, 390.
Koehn, C. J., 137.
Kögl, F., 379, 387, 388.
Kohn, S., 31.
Koks, M. T., 170.
Kon, S. K., 60, 74, 223.
Koolhaas, D. R., 241.
Korsakoff, S., 44, 45, 53.
Krause, M. E., 137.
Krebs, H. A., 342, 343, 347, 348, 354, 355.
Krishman, B. G., 224.
Kubli, V., 217, 225.
Kudrjawzewa, A., 368.
Kuhn, R., 132, 136, 179.
Kühnau, J., 14, 105, 241.
Kuthy, A. V., 389.

Lajos, S., 326, 329.
Lal, R. B., 25, 33.
Landis, H. R. M., 8, 14, 55, 67, 76.
Laquer, F., 143, 156.
Larue, G. H., 40, 51.
Lasch, F., 54.
Layani, F., 52.
Lease, J. G., 137.
Leclef, G., 388.
Lecoq, R., 266, 277, 341, 353.
Ledoux, E., 52.
Lehmann, J., 364, 370.
Lelesz, E., 319, 329.
Leong, P. C., 80, 83, 211, 215, 223, 232, 241, 248, 250, 255, 297.
Lepkovsky, S., 4, 77, 136, 137, 267–270, 272–275, 278, 279.
Lerman, J., 30.
Lettsom, J. C., 34, 50.
Lewinson, M. S., 328, 330.
Libert, E., 31.
Light, R. F., 222.
Lillie, R. D., 136.
Ling, H. W., 215, 221, 224.
Lipmann, F., 161, 171, 342, 345, 346, 348–350, 354, 355.
Lipschitz, M. A., 178, 355.
Little, J. M., 33.
Litzinger, A., 190.
Loewenthal, L. J. A., 14.
Lohmann, K., 72, 77, 172, 178, 337, 338, 344, 345, 350, 354.
Long, M. L., 30, 298.
Longcope, W. T., 54.
Loofbourow, J. R., 171.
Lowndes, J., 241.
Lu, G. D., 20, 32, 71, 77, 341, 353.
Lufkin, N. H., 41, 51.
Luikart, R. R., 42, 52.
Lurie, L. A., 54.
Luros, G. O., 135.
Lyman, C. M., 387.

MacArthur, E. H., 222.
Macgowan, 17, 27.
Mackie, T. T., 13, 31, 47, 53, 54.
MacLeod, F. L., 249, 255.
MacLeod, K., 25, 32.
Macy, I. G., 30, 292, 298.
McAlpine, D., 54.
McCann, W. S., 13.
McCarrison, R., 3, 13, 59, 60, 68, 72, 74, 120, 359, 367.
McClosky, W. T., 361, 368.
McCollum, E. V., 3, 13, 120, 127, 129, 171, 217, 225, 283, 297.
McCosh, S. S., 298.
McDonald, W. J., 42, 52, 71, 77.
McGowan, G. K., 343, 350, 354.
McHargue, J. S., 361, 368.
McHenry, E. W., 137, 274, 279, 283, 297, 346, 347, 355.
McLaughlin, A. J., 63, 75.
McLester, J. S., 13, 53, 120.
McNiele, O., 30, 71, 76.
McVicar, C. S., 31.
Madden, R. J., 136.
Makino, K., 174, 179.
Malcolm, J., 171.
Malcolmson, J. G., 17, 27.
Maliwa, E., 33.
Malmberg, M., 137.
Mann, P. J. C., 211, 224.
Mann, W. L., 25, 32.
Mapson, L. W., 211, 224, 367.
Marks, H. E., 47, 55.
Marrian, G. F., 337, 339, 347, 352, 368.
Martin, R. W., 78, 328, 330.
Mason, V. R., 55.
Masuko, R., 359, 367.
Mathews, A. P., 120.
Mattill, H. A., 137.
Matsumura, S., 28.
Maudsley, C., 211, 223.
Maurer, S., 292, 298.
Mayer, A., 69, 76, 367.
Mayer, M. B., 33.
Means, J. H., 30.
Mcbius, J., 66, 68, 75.
Meiklejohn, A. P., 87, 93, 220, 225, 249, 255, 353.
Mellanby, E., 64, 75, 120.
Mendel, L. B., 59, 74.
Mengert, W. F., 41, 52, 71, 77.
Messer, F. C., 76.
Meyer, A., 35, 50.
Meyerhof, 336, 363.
Michaelis, L., 162, 171.
Miyadera, K., 361, 368.
Miller, D. K., 53, 54.
Miller, F. R., 137.
Miller, W. L., 387.
Mills, W. H., 171.
Minot, G. R., 13, 34, 35, 47, 50, 53, 54.
Mitchell, A. G., 13.
Mitchell, H. H., 130, 135, 276, 279.
Miura, K., 65, 75.
Miyata, R., 28.
Molitor, H., 79–83, 361, 368.
Moll, T., 207, 209, 215, 223.
Monaghan, B. R., 365, 370.
Moore, B., 142.
Moore, C. N., 358, 366.
Morgan, A. F., 76, 137, 161, 170.
Morgan, B. G. E., 215, 221, 224.
Morros Sarda, J., 368.
Moskowski, M., 29.
Muller, H., 32.
Munsell, H. E., 242.
Murakoshi, Y., 17, 28.
Murphy, E. A., 269, 278.
Musser, J. H., 13, 53.
Myers, W. K., 92.

Nagai, S., 350, 362, 368, 369.
Nagays, M., 28.
Naiman, B., 171.
Naito, H., 29.
Nakahara, W., 255.
Nelson, E. M., 222, 242.
Nelson, V. E., 234, 242, 387.
Neuberg, 341, 343, 346.
Neumann, H., 15, 40, 51.
Nocht, 32.
Noguchi, K., 28.

O'Brien, J. R., 136, 137, 143, 156, 171, 223, 353.
Ochiai, S., 28.
Ochoa, S., 345.
Oeriu, S., 361, 368.
O'Flynn, E., 54.
Ogata, T., 28.
Ogata, T. S., 75.
Oh, H. Y., 369.
Ohdake, S., 139, 140, 142, 143, 156, 161, 170, 215, 224, 306.
Ohomori, K., 29.
Ohta, K., 358, 366.
Oka, C., 28.
Oka, H., 28.
O'Leary, P. A., 31, 54.
Orr-Ewing, J., 389.
Osterberg, A. E., 69, 76.
Outhouse, J., 30, 298.
Overbeek, G. A., 366.

Palladin, A., 368.
Palmer, L. S., 385, 390.
Parade, G. W., 211, 224.
Park, F. S., 33.
Parker, H. B., 33.
Parsons, H. T., 137, 274, 279, 379, 388.
Passmore, R., 71, 77, 352.
Patek, A. J. Jr., 50, 54, 56.
Patterson, A. B., 33.
Pavcek, P. L., 235, 242, 387.
Pearse, F., 25, 33.
Perla, D., 81, 83, 286, 287, 297.
Peskett, G. L., 382, 387.
Peters, B. A., 14.
Peters, J. P., 26, 33, 59, 74.
Peters, R. A., 3, 13, 71, 77, 139, 141, 143, 156, 171, 172, 174, 178, 208, 215, 222-224, 337-344, 350-355, 358, 362, 364, 369, 382, 389.
Peters, W., 389.
Peterson, W. H., 235, 242, 387, 389.
Pflimlin, R., 15.
Phipard, E. H. F., 119.
Pickel, F. D., 190.
Pilcher, F., 69, 76, 367.
Pilcher, J. D., 258, 265.
Pilcher, R. W., 276, 279, 290, 298.
Pines, I., 80.
Pi-Suñer Bayo, P., 319, 329.
Pitz, W., 283, 297.
Plass, E. D., 41, 52, 71, 77.
Platt, B. S., 20, 30, 32, 71, 77, 341, 353, 357.
Plimmer, R. H. A., 228, 241, 258, 265.
Plummer, N., 76.
Plymate, H. B., 358, 366.
Polak, J. J., 344, 354.
Poljakoff, W., 31.
Polland, W. S., 51.
Poole, M. W., 298.
Porter, W. B., 66, 75.
Portier, P., 385, 390.
Potter, V. R., 178.
Pound, R. E., 13, 31, 47, 54.
Prebluda. H. J., 171, 217, 225.
Prickett, C. O., 219, 225.
Prinzing, F., 26, 33.
Pritchard, W. H., 137.
Putnam, J. J., 32.
Pyke, M. A., 225.

Quinn, E. J., 235, 242.

Ragle, B. H., 27, 31.
Ramoino, 347.
Randoin, L., 266, 319, 329, 362, 369.
Rau, S., 367.
Raymond, W. H., 241, 258, 265.
Reader, V., 223, 276, 279, 382, 389.
Record, P. R., 242, 389.
Reed, A. C., 13, 33, 53.
Retuiger, J., 33.
Rhoads, C. P., 53, 54.
Richardson, L. R., 137, 189, 388.
Richter, C. P., 298.
Riddell, J. D., 29.
Rigler, R., 285, 297.
Robbins, W. J., 180, 181, 189, 193, 195, 379, 384, 385, 388, 389.
Robertson, E. C., 224.
Robinson, R., 190.
Roehm, R. R., 220, 225, 387.
Rogers, C., 17, 27, 47.
Rogers, M. H., 47, 55.
Rogoff, J., 261, 265.
Romano, J., 38, 51.
Roosevelt, J. W., 32.
Root, H. F., 47, 55.
Roscoe, M. H., 202, 203, 214, 216, 222, 224, 231, 235, 241, 250, 255.
Rose, M. S., 119, 120.
Rosedale, J. L., 258, 265.
Rosenberg, A., 71, 77, 364, 370.
Rosenberg, H. A., 261, 265.
Rosenthaler, L., 171.
Ross, J. R., 76.
Rowe, A. W., 265, 298.
Rowlands, M. J., 60, 74, 389.
Roy, S. C., 25, 33.
Roy, W. R., 361, 368.
Rubino, P., 319, 329.
Rudy, H., 179.
Ruehle, A. E., 156, 157, 171, 189.
Ruhkopf, H., 143, 156.
Rumpel, T., 33.
Ruschke., 14.
Russell, W. R., 40, 51.
Rydin, H., 354.

Saleeby, N. M., 263, 265.
Salmon, H., 179.
Salmon, W. D., 222, 269, 274, 278, 360, 367.
Sample, A. B., 30.
Sampson, W. L., 79–83, 211–215, 224, 285, 297, 326, 361, 368.
Sanchez-Rodrigues, J., 368.
Sankaran, G., 224.
Sasaki, R., 242.
Sato, F., 28.
Saunders, D. H., 220, 225, 387.
Schaumann, 197.
Scheunert, A., 222, 232, 235, 241, 242, 367, 389.
Schieblich, M., 222, 232, 241, 242, 388, 389.
Schioedt, E., 32.
Schiro, H., 49, 55.
Schittenhelm, A., 26, 33.
Schlecht, H., 26, 33.
Schlenk, F., 137.
Schlitter, E., 179.
Schmitz, E., 362, 369.
Schneider, A. J., 54.

Schonborn, V. E., 318, 329.
Schopfer, W. H., 180, 189, 195, 220, 225, 375, 376, 378, 381, 383, 384, 387, 389, 390.
Schopp, K., 179.
Schoubye, N., 222, 359, 367.
Schrader, G. A., 219, 225, 323, 325, 329, 341, 347, 353.
Schroeder, H., 105, 241.
Schultz, A., 220, 225, 387.
Schultz, F., 143, 156.
Schultz, H. W., 137.
Schuster, Ph., 72, 77, 172, 178, 337, 338, 344, 345, 354.
Schwochow, P., 14.
Sciclounoff, F., 15, 39, 48, 51.
Scott, L. C., 66, 75.
Sebrell, W. H., 53, 58, 73.
Seidell, A., 139, 140, 142, 143, 206, 207, 214, 222, 224.
Selfridge, G., 92.
Sell, M. T., 222.
Shastid, T. H., 15, 92.
Shattuck, F. C., 32.
Shattuck, G. C., 34, 35, 50.
Sherwood, F. F., 387.
Sherman, H. C., 69, 76, 120, 159, 170, 202, 203, 216, 222, 232, 280, 282, 284–286, 311, 312.
Sherman, W. C., 341, 342, 353, 354.
Shimamura, T., 142.
Shimazono, J., 65, 75.
Shin, H., 247, 254.
Shindo, T., 370.
Shinobe, S., 361, 368.
Simmonds, N., 120, 283, 297.
Simola, P. E., 344, 354.
Simonnett, H., 266.
Simpson, G., 142.
Simpson, G. C., 29.
Simpson, G. K., 366.
Simpson, I. A., 69, 76.
Sinclair, H. M., 71, 77, 181, 189.
Sindoni, A. Jr., 55.
Skarzynska-Gutowska, M., 358, 366.
Smith, A. H., 298.
Smith, C. H., 29.
Smith, F. M., 67, 75.
Smith, M. E., 262, 265, 298, 355, 361, 367, 368.
Smith, M. I., 130, 131, 136, 169, 209, 214, 223, 224, 361, 368.
Smith, S. L., 120.
Smithburn, K. C., 53.
Sollman, T., 258, 265.
Solomon, P., 52.
Somekawa, E., 255.
Spies, T. D., 11, 13, 14, 15, 33, 36, 38, 45, 49, 51, 53, 55, 74, 75, 81, 83, 136.
Sparks, M. I., 76, 358, 366.
Spruyt, J. P., 104, 105, 229, 230.
Stähler, F., 43, 52, 71, 77.
Stanley, A., 75.
Stanton, A. T., 109, 120, 125, 126, 129, 138, 142, 295, 305, 308.
Stanton, H. T., 28
Steenbock, H., 222, 247, 254, 273, 278, 389
Steinberg, C. L., 92
Stepp, W., 15, 105, 127, 129, 24
Stern, K. G., 178, 354.
Stevens, J. R., 136.
Stewart, C. P., 370.
Stiebeling, H. K., 109, 119.
Stitt, E. R., 30, 75, 120.
Stone, L., 57, 73.
Strauss, M. B., 13, 30, 31, 35, 42, 51, 52, 54, 65, 71, 75, 77.
Street, H. R., 390.
Strong, F. M., 136.
Strong, R. P., 29, 306, 308.
Sturgis, C. C., 54.
Sturm, A., 30.
Sugiura, K., 171.
Sumnerfeldt, P., 76.
Sunderlin, F., 379, 388.
Sure, B., 30, 60, 67, 69, 74, 76, 79, 83, 120, 262, 265, 274, 279, 289, 294–299, 355, 357, 360, 361, 365, 366–368, 370.
Suzuki, K., 369.
Suzuki, M., 28.
Suzuki, S., 28, 142.
Suzuki, T., 28.
Suzman, F. M., 54.
Sweetman, M. D., 385, 390.

Takahashi, N., 244, 254.
Takaki, K., 28, 124, 128, 295.
Takamatsu, A., 370.
Takamatsu, M., 367.
Takata, Y., 365, 370.
Tanikawa, K., 28.
Tanner, W. F., 136.
Tarr, E. M., 30, 71, 76.
Tatum, E., 389.
Tauber, H., 166, 171, 178, 345, 352, 355.
Taylor, F. H. L., 341, 353.
Taylor, F. R., 53.
Thatcher, H. S., 60, 74.
Theobald, G. W., 42, 43, 52.
Thompson, R. H. S., 341, 353.
Thomson, D., 29.
Thornton, J. J., 143, 225, 313, 315.
Tisdall, F. F., 13, 76.
Tislowitz, R., 79, 80, 83, 360, 368.
Todd, A. R., 154–156, 161, 170, 171, 174, 179, 189, 190, 193, 196, 385.
Tolpin, J. G., 195.
Tomey, L. F., 273, 279.
Tomlinson, M. L., 190.
Tonnis, B., 387.
Tosatti, E., 361, 368.
Truesdail, J. H., 387.
Tsai, L. S., 292, 298.
Tschesche, R., 143, 147, 156, 190, 196.
Tsunoo, S., 367.
Tuilant, A., 41, 52.
Turner, R., 298.

Ueno,, J., 60, 75.
Uga, Y., 72, 77, 361, 369.
Ungar, A., 368.
Ungley, C. C., 54, 78.
Urmy, T. V., 27, 31.

Van Berkhaut, 347.
Vandervelde, P., 33.
Van Klaveren, F. W., 179.
Van Leersum, E. C , 129.
Van't Hoog, E. G., 385, 390.
Van Veen, A. G., 103, 105, 170, 202, 221, 228, 241.
Vaughan, J. M., 78.
Vedder, E. B., 23, 24, 29. 30, 32, 58, 73, 75, 105, 120, 125, 126, 129, 139, 142, 159, 170, 221, 257, 258, 264, 312.
Vestin, R., 179.
Vetter, H., 179.
Vogt-Møller, P., 363, 364, 370.
Von Drigalski, V. W., 72, 78.
Vorderman, A. G., 28.
Vorhaus, M. G., 14, 39, 43, 48, 51, 72, 76, 78, 93.

Wagener, H. P., 92.
Wagner-Jauregg, Th., 136, 179.
Wahoyama, H., 242.
Walker, D. J., 294, 298, 299.
Walshe, F. M. R., 30.
Warburg, O., 162, 170.
Ward, A., 298, 357, 366.
Waring, J. I., 32.
Warner, G. C., 389.
Waterman, R. E., 14, 17, 27, 39, 43, 48, 51, 72, 76, 78, 143, 156, 169–171, 189, 204, 208, 209, 215, 222–224, 232, 253, 278, 280, 284, 285, 287, 297, 301, 304, 308, 311, 326.
Watson, C. J., 55.
Webster, A., 142.
Webster, D. R., 358, 366.
Wechsler, I. S., 35, 41, 47, 50, 52.
Weese, H., 79–83.
Weil, M. P., 31.
Weill-Hallé, B., 52.
Weiss, S., 21, 32, 53, 59, 63, 65–67, 74, 75, 341, 353.
Weller, G. L. Jr., 31.
Wells, A. H., 93.
Wenckebach, K. F., 23, 32, 65, 66, 75, 120, 285, 297.
Went, F. W., 389.
Werkman, C. H., 379, 388.
Wertheimer, E., 101, 105, 275, 276, 279, 320, 321, 326, 329.
Westenbrink, H. G. K., 207, 217, 223, 225, 244–247, 254, 256, 258, 261, 264, 265, 269, 272, 278, 318, 323, 329, 337, 340, 344, 352-354, 364–366, 370.
Westphal, K., 154–156, 179, 189, 196.
Whalen, F. B., 235, 242.
Wheeler, G. A., 136.
Wheeler, W. A., 33.
Whipple, D. V., 274, 278, 346, 355.
White, P. R., 379, 388.
Whitfield, D. W., 41, 52.
Wilbur, D. L., 14.
Wilder, R. M., 14, 81, 83.
Wildiers, 373.
Wilkins, R. W., 21, 32, 59, 63, 65, 66, 74, 341, 353.
Wilkinson, B., 389.
Willcox, W. H., 29.
Williams, R. J., 220, 225, 373, 387.
Williams, R. R., 14, 17, 27, 29, 38, 39, 43, 48, 51, 72, 76, 78, 105, 139, 140, 143, 154–157, 170, 171, 179, 189, 196, 206, 207, 213, 222–224, 263, 265, 278, 283, 297.
Wills, L., 367.
Windaus, A., 140, 143, 146, 147, 156, 190, 196.
Wintersteiner, O., 156.
Witts, L. J., 78.
Wohmann, M., 156.
Wolbach, S. B., 13, 74.
Wood, C., 77.
Woods, H., 389.
Woollard, H. H., 368.
Woolley, D. W., 136.
Wright, M. D., 105, 109, 119, 160, 170, 212, 224, 232, 241.
Wright, S., 366.

Yacoub, K., 30.
Yamagishi, T., 156, 161, 170, 215, 224.
Yang, C. S., 30, 31, 63, 75.
Yonechi, S., 274, 279.
Youmans, J. B., 13.

Zentmire, Z., 14.
Zerfas, L. G., 43, 46, 52, 54.
Ziskind, E., 55.
Zschiesche, E., 11, 15.
Zwaardemaker, H., 285, 297.

INDEX OF SUBJECTS

Absorption from intestine, 4, 18, 27.
Absorption, ultraviolet. *See* ultraviolet absorption.
"Accessory" substances, 3, 126. *See also* vitamins, category of.
Acetaldehyde, 338, 350.
Acetic acid, 345–349.
Acetylation of thiamin, 165.
Achlorhydria, 5, 13, 35, 59.
Acidity, effect on thiamin. *See* pH.
Acrodynia preventing factor, 134.
Activation of carboxylase, 345.
Adenylic acid, 211.
Administration of thiamin, 79–82, **89.**
Adsorbents. *See* charcoal, fuller's earth, and zeolite.
Alcohol group of thiazole, 173.
  oxidation in tissues, 350.
Alcoholic addicts, 21, 34–40, 85.
  fermentation, 335, 374.
  neuritis, treatment, 38.
  pellagra, 45.
  polyneuritis, 12, **34–40,** 61.
Alimentary tract, 8. *See also* gastro-intestinal.
Alkali, action on thiamin, 162–165.
  sensitivity of thiamin, 158–165.
American diets, 107.
Amino acetanilide, 165, 166, 217.
  acetophenone, 165–166, 217.
  group in thiamin, 174–175.
  pyrimidines, 149–151, 183.
  sulphonic acid, 146, 147, 152, 154.
Anaerobic glycolysis, 342.
Analogues of pyrimidine intermediate, **180–190.**
  of thiamin, **172–179.**
  of thiazole intermediate, **191–196.**
Analysis of thiamin, **167–168.**
Anastomosis, intestinal, 27.
Anemia in beriberi, 22.
  in pregnancy, 42.
  preventing factor, 134.
Anemias, 5, 12, 13, 26, 42, 72.
Anesthesia, 21, 23.
Aneurin, 4, 134. *See also* thiamin.
Animal tissues, as sources of vitamin B_1, 228, **236.**
  storage in, **243–250.**
Anorexia, 5, 8, 22, 57, 69, 86, 95, 102, 261, **289–290,** 357.
Anti- as prefix in vitamin names. *See* animal species affected or pathological condition involved.
Aphonia, 22.
Appetite, loss of. *See* anorexia.
  restoration method of assay, 198, **206–207.**
Appraisal of Oriental diets, **94–104.**
  of particular diets, 110.
Arrhythmia, 80.
Arthritis, 87.
Ascites, 21.
Ascorbic acid. *See* vitamin C.
Assay for vitamin B_1. *See* assay methods.
Assay methods, 87, **197–225.** *See also* various animal species and principles, *e.g.*, protective, curative, growth, etc.
  accuracy of, 199, 219, 220.
  reproducibility of, 199, 219, 220.
Associated pathological changes, 6.
Asthenia, 26.
Ataxia, 21.
Atrophy of intestines, 69.
  of muscles, 21.
Auerbach's plexus, 69.
Auxins, 371.
Avian beriberi. *See* polyneuritis gallinarum.
Avitaminosis. *See* specific deficiency diseases.
Azo test. *See* formaldehyde azo test.

Bacterial synthesis, 314, 378.
Balance among vitamins, 3.
Beans. *See* legumes.
Beef. *See* meat.
Beetles, need for thiamin, 385.
Benzoylation, 140, 165.
Beriberi, **16–24.** *See also* special aspects, *e.g.*, geographical distribution, pathology, treatment, etc.
  correlation with weight, **306–307.**
  diseases related to, 24–27.
  pathology, **57–73.** *See also* special aspects.
  prevention, 28, 29, 86. *See also* prevention.
  sporadic, 26.
  symptoms, **19–23.**
  synonyms for, 16.
  treatment of, 17, 24, 28, 86.
"Beriberi heart," 21, **61–63.**
Beverages, vitamin B_1 in, 240.
Biochemical lesion, locus of, 338.
Bios, 4, 373.
Biotin, 371, 373.
Bisulfite-binding substances, 340, 341.
Black-tongue factor (dog), 4, 131–134. *See also* nicotinic acid.
Blood, assay of vitamin B_1 in, 87, **220.**
  chemistry, 360–361.
  leucocytes, 363.
  sugar, 79, 316–330, **323.**
Blood pressure, 80.
Body fat, 346.
  weight, maintenance of, 70, 261, **300–303.**
Borderline states, 3–4, 64.
Botanical species, thiamin in, 375–378.
Bradycardia, 26, 80.
  and inanition, 211.
  method of assay, 198, 199, **210–213.**
Brain tissue, pyruvic acid in, 338–339.
Bread, **231–233,** 238.
Breast milk. *See* milk, human.
Butter, vitamin B_1 in, 229, **237.**

Cachexia, 26.
Cancer, 50, 61, 85, 250.

Capillary permeability, 79.
Carbohydrate metabolism, 80, **331–355.**
  clinical studies of, **71–72.**
  in all cells, 332–334.
Carboxylase. *See also* cocarboxylase.
  activation of, 345.
Cardiac beriberi, 16.
  dilatation, 16, **63–67.**
  enlargement, 16, 19, 20, 21, 23, 59, **62–63,** 285
  failure, 8, 23, 24, 62–63, **64–67,** 89.
  failure and edema, 65.
Cardio-respiratory symptoms, 21, 65.
Cardiovascular failure, mechanism of, **64–67.**
  symptoms, 5, **8,** 21, 23, **59, 61–63, 64–67.**
Castle's extrinsic factor, 73, 134.
Cataract preventing factor, 132, 133.
Catatorulin action of vitamin B_1, 337–340.
Cattle, vitamin synthesis, 378.
Celiac disease, 3, 31, 61.
Centrifuge technique, 140–142.
Cereals, vitamin B_1 in, 229, **238.**
Cevitamic acid. *See* vitamin C.
Charcoal as an adsorbent, 139, 140.
Chase-Sherman unit, 169, **213–216.**
Cheese, vitamin B_1 in, 229, **237.**
Chemical derangements, **356–370.**
  features of thiamin, 145.
  methods of assay, 87, 198, 199, 216–220.
Chick dermatitis, 132, 134.
  method of assay, 197, **202.**
Chick-Roscoe unit, 202–203.
Children, response to vitamin B_1 rich diets, 291–292.
  susceptibility to deficiencies, 5, 71.
"Chloroxy" vitamin, 165, 175.
Cholesterol, 365.
Choline, physiological role of, 134, 274, 283, 284, 346.
  chemistry of, 145.
Cirrhosis of liver, 50, 61.
Citric acid, 343.
Claims, allowable for thiamin, 86.
  of thiamin content, 90, 91.
Cleavage of thiamin, 146, 147.
Clinical response to vitamin B_1 therapy, 90.
Clinical studies with crystalline vitamin B_1, 71.
  in alcoholic polyneuritis, 38.
  in diabetes, **48.**
  in Korsakoff's syndrome, 39.
  in polyneuritis of pregnancy, 43, 71.
Clinical indications for thiamin therapy, 86.
Cocarboxylase, 172, 173, 238, 343, **344–346.**
  See also thiamin pyrophosphate.
  determination of, 345.
  in brain tissue, 344.
Coenzyme function of thiamin, 331–351.
Coenzymes, 336.
  relation of vitamins to, 132.
Coexistence of deficiency diseases, 24.
Coexistent diseases, treatment of, 90.
Colitis, 47, 61, 85.
Color reactions of thiamin, 165–166.
Congo red, 79, 360.
Conversion of units, 91.
Cooking processes, loss of thiamin in, 85, 158, 160, 227, 230–233.
Co-ordination, lack of, 21.
Coprophagy, 203.
Council of Pharmacy, allowable claims for thiamin, 86.
Cowgill formula, 94–104, **300–315.**
Cowgill's values reappraised, **95–99.**
Cozymase, 336.
Cramps, 20.
Craving for vitamin B_1, 289.
Crystalline vitamin B_1. *See* clinical studies with.
Curative methods of assay, 198, **206–210.**
Cures, spontaneous, 208.
Cyanosis, 19, 20, 21, 23.
Cytochrome, 336.

Dairy products, vitamin B_1 in, 229, **237.**
Day-dose principle, **208–209.**
Decarboxylation, 337–338, 342–346.
  and oxidation, **345–346.**
Decortication of grains. *See* grains, milling of.
Deficiency, degree of, correlated with symptoms, 20.
Deficiency diseases, pathology of, 5.
Definition of "vitamin," 128.
Degeneration, neural, 57–58. *See also* nervous system lesions.
  of spinal cord, 5.
Degree of deficiency in relation to symptoms, 20.
Dehydrogenases, 335–337.
Depletion of vitamin B_1 in tissues, **244–248.**
Dermatitis. *See* skin lesions, rat dermatitis, chick dermatitis, turkey dermatitis.
Determination of vitamin B_1. *See* assay methods.
Diabetes, **47–48, 72,** 85, 328.
  polyneuritis of, 12, **47–48,** 61.
Diabetic diets, 48.
Diabetic tabes. *See* diabetes, polyneuritis of.
Diagnosis of beriberi, 19–23.
  of vitamin B_1 deficiency by urinary analysis, 87, **102,** 217–218, 250–253.
Diamino pyrimidine from thiamin, 153–154.
Diarrhea, 4, 5, 8, 22.
Diazo reagent, 165–166, 198, 217.
Dietary habits, 7, 13, 22, 90.
  precautions, 84–85.
Diet in organic diseases, 85, 90. *See also* organic diseases.
Diets, appraisal of, 110.
  beriberi preventing, 95.
  beriberi producing, 22, 27, 95.
  deficient in thiamin, 90.
  effective in treating thiamin deficiency, 88. *See also* diets, therapeutic.
  human in relation to beriberi, **94–103.**
  low fat, 101.
  low protein, 101.
  Occidental, 101, **106–119.**
  of American families, 107.
  Oriental, appraisal of, **94–104.**
  reducing, 111, 117, 118.
  therapeutic, 88, 90.
  therapeutic, thiamin content of, **111–118.**
Differentiation of the vitamins, 127–128.
  of vitamin B, **130–137.** *See also* frontispiece.
Digestion, 358–360.
Dimorphism, 140, 153, 166.
Diphtheria, 14.
Discovery of vitamins, **123–129.**
Dismutation, 342, 347–348.
Distribution of thiamin in foods, **226–242.**
  in plants, 373–378.
Diuretic properties, 79.
Dosages of supplements, 88.
  of thiamin, 88, 89.
Doses, lethal, 81.
Dropsy, epidemic, **25.**
Drosophila, 385–386.
Drugs, action in thiamin deficiency, 361.
Dry beriberi, 16, 19, 68.

Dyspnea, 8, 19, 20, 21, 24, 102.
Dystrophy. *See* gastro-intestinal disturbances.

Edema, 5, 6, **8**, 19, **21–22**, **59**, **63**, **67–68**, 87.
and cardiac failure, 65.
mechanism of, **67–68**.
"Edema disease," 25.
Edemas, various, 26.
Egg white injury factor, 134.
Eggs, vitamin B_1 in, 229, **237**.
Electrocardiographic changes, 18, 66, 80, 102, 211.
Elution of thiamin, 140, 213.
Embden-Meyerhof theory, 334–335.
Endocrines, 60, **73**, 360–361.
Enzyme, yellow respiratory, 132.
Enzymes, 4, **335–336**.
relation of vitamins to, 132.
Enzymic disturbances, 358–360.
Epidemic dropsy, **25**.
Epigastric distress, 20.
Epilepsy, 80.
Epithelial tissues, lesions of, 5. *See* skin lesions.
Esters of thiamin, 173.
Ethers of thiamin, 173.
Etiology of beriberi, 18, 29.
Evaporation technique, 140–142.
Excretion of vitamin B_1, 102, **250–253**.
Exercise, 4, 19, 261–263, 340.
Extrinsic factor, Castle's, 73, 134.

Factor I. *See* vitamin B_6.
Family incidence of beriberi, 22.
Fatigue, 19, 102.
Fat-soluble vitamins, history, 127.
Fat synthesis and vitamin B_1, 273, 274, **346–348**.
Fats, vitamin B_1 in, 240.
vitamin B_1 sparing action of, 101, 108, **266–276**.
Fatty acid esters, influence on vitamin B_1 requirement, **268–271**.
Fatty acids and vitamin B_1 requirement, 275.
Fatty livers and vitamin B_1, 283.
Feces, vitamin B_1 in, **250–253**.
Ferments. *See* enzymes.
Fever, 4, 19, 25, 85, 90, 264.
Filtrate factor, 4, 134.
Fish, vitamin B_1 in, 236.
Flavobacterium vitarumen, 378.
Flour, white, 24, 109, **231–232**, 304–305.
Fluorescence test for thiamin. *See* thiochrome test.
Foods, classification by thiamin content, 109.
distribution of vitamin B_1 in, **226–242**.
enrichment with thiamin, 104.
low in thiamin, 110.
miscellaneous, vitamin B_1 in, **234**, 240.
rich in thiamin, 85, 88, 109.
tables of thiamin content, **236–240**.
thiamin content of average servings, 112–115.
Forced feeding, 200.
Formaldehyde, action on thiamin, 165.
azo test for thiamin, 165, 198, 216.
Formula of thiamin, empirical, 146.
of thiamin, structural, 144
Fowl method of assay, 197, 200.
Fruits, vitamin B_1 in, 233.
Fuller's earth as an adsorbent, 139, 140.
Functional groups of pyrimidine, 181–183.
of thiamin molecule, 172–179.
of thiazole, 191–193.
Fungi, need for thiamin, **381–382**.
thiamin in, 378, **381–382**.

Gallbladder, 31.
Gastric dysfunction, 69.
secretion in pregnancy, 42.
Gastro-intestinal disturbances, 4, 13, 15, 21, 31, 32 **59–60**, **68–70**, 85, 86, 89, 90, 358–360.
General role of thiamin, **371–390**.
Generalized function of thiamin, **331–334**.
Geographical distribution of beriberi, 17.
Germination of seeds, 348, 380–381.
Glandular disturbances, 00, 73. *See also* endocrines.
Glaucoma in epidemic dropsy, 25.
Glossitis, 6.
Glucose, conversion to lactic acid, 316–317.
Glutathione, 362.
Glycerol and vitamin B_1 requirement, 275.
Glycogen, liver, in relation to vitamin B_1, 275, **313–330**.
storage, **316–330**.
Glycogenolysis, 316–317.
Glycolysis, 316–317, 334–335.
Glycosuria, 328.
Glyoxalase, 341.
Grains, milling of, 85, 103, 104, 106, 109, **229–233**. *See also* milling.
Gray hair preventing factor, 134.
Green vegetables, 233.
Growth, 4, 22, 70, 86.
and vitamin B_1 intake, **280–284**, 290–291.
methods of assay, 197, **202–205**.
Growth of microorganisms for assay of vitamin B_1, 198, **220**.
influenced by pyrimidines, 180–182.
and thiamin, 371–390.

Heart. *See also* cardiac.
automotive, 285.
block, 211.
effect of B_1 on, 80.
in vitamin B_1 deficiency, **62–63**, **64–67**.
pathology of, 62–63.
rate, 80, 199, 210–212, 285.
right-sided failure, 21, **65**.
"Heart disease," nutritional. *See* cardiovascular symptoms.
Hematopoiesis, 5, **72**.
Hemoglobin, 6.
Hemorrhage, 5, 25.
Herpes zoster, 14.
History of beriberi, 17, 27–33.
of vitamins, early, **124–129**.
Hofmann degradation, 155.
Hormones. *See* endocrines.
Hunger edema, 26.
Hydrogen in position 2 of thiazole, 175.
Hydropericardium, 21.
Hydrothorax, 21.
Hydroxyl group of thiazole, 173.
Hyperesthesia, 102.
Hyperglycemia, 324–328.
Hyperkeratosis. *See* skin lesions.
Hyperthyroidism, 4, 19, 85, 86, 90, 262, 360–361.
Hypochlorhydria, 35, 59.

Idiosyncrasies, 3, 19, 22.
Inanition, 337, 356–357.
Incidence of beriberi, 17, 263–264.
Increased requirement of thiamin, **18–19**, 22, **85–86**.
Indications for thiamin therapy, 86.
Indophenol, 336.
Infantile beriberi, 5, 19, 23, 57, 63, 89.
Infants, susceptibility to deficiency, 5, 13, 71, 290–293.
Infection, 86.
Inflammatory processes, 79.

Injection method of assay, **206–210.**
of thiamin, 89. *See* clinical studies with crystalline vitamin B_1.
Insects, need for thiamin, 385–386.
Insomnia, 4, 19.
Insulin and vitamin B_1, **324–328.**
Intercurrent disease, 7, 22, 295–296.
Intermediate carbohydrate metabolism, **331–355.**
Intermediates of thiamin, action on animals, 176, 180.
action on plants, 180, 383–384.
Internal secretions. *See* endocrines.
International standard clay, unavailability of vitamin in, **213–216.**
International unit, 168–169, **203, 213–216.**
Interrelationships among vitamins, 3, 328. *See* balance among vitamins.
Intestinal dysfunction, 69–70. *See also* gastrointestinal disturbances.
motility, effect of thiamin on, 81, 86.
Intramuscular injection, 79–82, **89.**
Intravenous injection, 39, 79–82, **89.**
Iron preparations, 6.
Isolation of thiamin, **138–143.**

Jamaica ginger extract poisoning, 11.
Jansen and Donath isolation, 139.

Ketoglutaric acid, α, 343.
Korsakoff's syndrome, 12, 39, 42, **44,** 61.

Lactation and vitamin B_1, 4, 19, 30, 70, 81, 85, 286, 287, **293–294.**
Lactic acid bacteria, 345.
Lactic acid, effect on polyneuritis, 324, 341.
from glucose, 316–317, 334–335.
in brain tissue, 338.
metabolism in avitaminosis, 323–324.
Lactic dehydrogenase, 340, 342.
Lactoflavin, 4. *See* riboflavin.
Leaves, thiamin in, 375–378, 380–381.
Legumes, vitamin B_1 in, 229, **237.**
Lesion, locus of biochemical, 338.
Lethal doses, 81.
Lipide metabolism, 364–365.
Liver fat, 346.
glycogen, 313–330.
hormone, 363.
preparations, 6, 36, 46, 92.
Loss of thiamin in cooking processes. *See* cooking processes.
Low-protein diet, 26.

Macrocytic anemia, 30.
Malaria in epidemic dropsy, 25.
Malignancy, 50, 61, 85, 250.
Manure, thiamin in, 381.
Meat as source of vitamin B_1, 228, **236.**
Mental disturbances, 5, 8, 12, 21.
efficiency and vitamin B_1 intake, 291–292.
Metabolic rate, 19, 22, 261–264.
Metabolism, increased, 22.
Methods of assay for vitamin B_1. *See* assay methods.
Methyl glyoxal, 340, 341, **363–364.**
injection of, 364.
Methyl group in position 2 of pyrimidine, 176, **181.**
Methylene blue, 337.
Methylene bridge in thiamin molecule, 175.
Microorganisms and thiamin, 371–375, 378–379, 382.
growth influenced by pyrimidines, 180–182.
growth of, as a method of assay. *See* growth of microorganisms as a method of assay.
Milk, human, toxic substance in, 363.
vitamin B_1 content of, 102, **292–293.**
Milk, vitamin B_1 in, 229, **237.**
Milligram equivalent, 95, 169.
Milling of rice, 229–231.
of wheat, 231–232.
Mineral metabolism, 361–362.
Minerals in blood, 6.
Miscellaneous foods, vitamin B_1 in, **234,** 240.
Molasses, vitamin B_1 in, 234, 240.
Mold growth test for thiamin in blood, 87.
Mortality from beriberi, 23.
Mouse method of assay, 197, **204.**
Mucous membrane lesions, 4, 6.
Multiple deficiencies, 4, 7, 356–357.
Muscle glycolysis, 334–335.
Mutual effects of vitamins, 3.
Myocardial damage, 18.

Names, origin of, 126, 134, 135.
Nerve function, sensitivity to deficiency, 391.
Nervous disturbance and pyruvic acid, 340–342.
system lesions, 5, **9,** 20, 21, **57–59, 61–62.**
Nervousness, 8, 19.
Neuritis, 5, **9, 10,** 20, 86.
alcoholic, 12, 34–40, 86.
causes of, 10.
cruralis, 15.
mechanism of, **64.**
optic, 15.
peripheral, 20, 22, 23, 24, 86, 89. *See also* polyneuritis.
Neuritis bacteriotoxic, **10, 11.**
chemical, **10, 11,** 15.
toxic, 11.
Nicotinamide, 371.
Nicotinic acid, 45, 131, **133,** 134.
amide, 133.
Night blindness, 6.
Nomenclature, 126, 134, 135. *See also* names origin of.
Nonspecificity of thiamin, 331–334.
Numbness, 21, 24.
Nutrilites, 371–374.
Nuts, vitamin B_1 in, 234, 240.
Nystagmus, 81, 198, 213.

Occidental diets, 101, **106–119.**
Optic neuritis, 15.
Optimum health, 3, 280–299.
Oral administration of thiamin, 38–39, 79–82, 88–89.
Organic diseases associated with deficiencies, 7, 22.
diet in, 85.
Organs, distribution of vitamin B_1 in, **243–250.**
Oriental diets, appraisal of, **94–104.**
Oryzanin, 126. *See* thiamin and vitamin B_1.
Overmilled grains, 22.
Oxidase theory of thiamin action, 339.
Oxidases, 335–337.
Oxidation and decarboxylation, 344–346.
of thiamin, 146, 161.
Oxidation-reduction of thiamin, 161–162.
"Oxy" vitamin, 175.
Oxygen consumption, 79.
uptake, **338–339,** 342.

Pantothenic acid, 371, 373.
Paraesthesia, 20.
Parenteral effects of thiamin, 79–82.
Parenteral injection, indications for, 89, 90.
of concentrates, 36, 85, 86, 89.
of thiamin, 85, 86, **89.**

Pathology of deficiency diseases, 5.
Pathology of vitamin B_1 deficiency, **57–64.**
in animals, **57–60.**
in human beings, **60–64.**
Pea roots, 192, 380, 384, 385.
Pellagra, 3, 5, 6, **44–45,** 61, 85, 131, 133.
polyneuritis of, 12, 13, **44–45,** 86, 108.
Pernicious anemia, 26, **46,** 61, 85.
"Pernicious beriberi," 16, 20, 23, 28.
Peroxidase reaction, 363.
*p*H, effect on destruction of thiamin, 159–161, 164–165.
of tissues, 359.
Pharmacology of vitamin B_1, **79–81.**
Pharmacopoeia Board on methods of assay, 210.
Phenyl glyoxal, 364.
Phosphopyridine nucleotides, 162.
Phycomyces, 180–182, 191, 192, 382, 384.
method of assay, 87, 198, **220.**
Physiology, pathological, **64–73.**
Phytophthora, 180, 384.
Pigeon-curative method, 208, 209.
glycogen storage of, **318–322.**
methods of assay, 197, 200, 201, 206–209.
Pigmentation of skin, 6. *See* skin lesions.
Plant growth influenced by pyrimidines, 180–182.
Plants and thiamin, 375–382.
Pleural effusions, relief by tapping, 89.
Polyneuritis, **34–56.**
alcoholic, **34–40.**
and cirrhosis of liver, 50.
and colitis, 47.
and malignancy, 50.
and tuberculosis, 49.
of beriberi, relation to other neurites, 61.
of diabetes, 12, **47–49.**
of Korsakoff's syndrome, 44.
of pellagra, **44–45,** 86. *See also* pellagra, polyneuritis.
of pernicious anemia, **46.**
of porphyrinuria, 49.
of pregnancy, 12, **41–44,** 61, 70, 86.
of senility, 50.
of sprue, 45.
of undetermined origin, 86.
time of onset in various species, 312–314.
Polyneuritis gallinarum, 28, 124, 125.
Polyuria, 26.
Pork. *See* meat.
Porphyrinuria, **49,** 61.
Potatoes. *See* vegetables.
Potency of vitamin B_1 preparations, **90–92.**
Potentiometric titration, 148, 149, 163.
Poverty as cause of defective diets, 7.
P-P factor, 4, 131, 133, 134. *See also* nicotinic acid.
Precipitants for thiamin, 138, 139, 166.
Precipitation methods of assay, 198.
Precordial pain, 21.
Predisposing causes of deficiency, 7.
Pregnancy, 4, 12, 19, 22, **41–44,** 70, 85. *See also* polyneuritis of pregnancy.
Preparations of vitamin B_1, **90–92.** *See also* vitamin B_1.
Prevention of beriberi, 28, 29, 86. *See also* beriberi.
of thiamin deficiency, **84–86.**
Prodromal symptoms, 5, 8, 102.
Prognosis of beriberi, 23.
Prophylaxis against vitamin B_1 deficiency, 84–85.
Protective foods, 7.
methods of assay, 197, **200–202.**
Protein deficiency, 26.
Psychoses. *See* mental disturbances.
Pulse in beriberi, 19.
Purity of thiamin, 167, 168.
Pyrimidine as activator of carboxylase, 345.
cleavage products of thiamin, 146, 147, 180, 181.
derivatives, 180–190.
intermediate of thiamin, 147, 180, 181.
nucleus, 144, 147.
Pyrimidines associated with thiamin, **180–190.**
synthesis of, 147, 183.
Pyruvate oxidase theory, 343, 350–352.
Pyruvic acid as cause of symptoms, 340–341.
as precursor of fat, 346–348.
hydrazone, 341.
in brain tissue, 338–339.
in carbohydrate metabolism, 334–335.
injection of, **340–341.**
in tissues, 341.
Pyruvic dehydrogenase, 345–346.

Quaternary nature of thiamin, 148, 149.
Quinine elution, efficiency of, 213–216.
Quinine in isolation, 140.

Rat-curative method of assay, 198, 207–210.
dermatitis, 131, 132.
growth factor, 130–134.
methods of assay, 197, **202–205,** 207–213.
glycogen storage of, **323–326.**
Reactions of thiamin, 158–167.
Reducing diets, thiamin content of, 111, 117, 118.
Reduction of thiamin, 161–162.
Refection, 203, 378.
Refined foods, 3, 22. *See also* foods.
Reflexes, Achilles, 20.
Reflexes, patellar, 20, 23.
Regeneration of nerve damage, 24, 64.
Reproduction and vitamin B_1, 70, **285–287.**
Requirement during lactation, **293–294.** *See also* lactation and vitamin B_1.
human, 85, **102–103,** 106, 107. *See also* increased requirement.
Requirement in relation to carbohydrate intake, **266–267.**
fat intake, **266–276.**
metabolic rate, **261–264.**
protein intake, **276–277.**
quantity of food, **256–265.**
special circumstances, **280–299.**
species, **309–315.**
weight, **300–308.**
Respiration rate, 81.
Respiratory paralysis, 65.
Respiratory quotient, 79, 325, 343, 347.
Response, clinical, 90.
Riboflavin, 131, **132,** 133, 134, 145, 328, 350, 371.
thiamin and insulin, 328.
Rice, 103–104, 112, 124–125, 200–201, 229–231, 238.
bird method of assay, 139, 197, **201, 202.**
milling, 229–231.
polish, 139, 229–231, 238.
polish as exclusive diet, 283.
polish extract, 7, 88, 89.
vitamin B_1 in, **229–231,** 238.
Rickets, 3, 5.
Rigidity of body in beriberi, 23.
Role of thiamin, **371–390.**
Root growth, 192, 380, 383, 385.
Roscoe unit, 169, 202–203.

Salts of thiamin, 166.
Saturation with thiamin, 18.
Schizophrenia, 87.

Scurvy 3, 5, 127.
Sedatives, 88.
Seeds, thiamin in, 348, 380–381.
Senility, neuritis of, 50, 61, 85.
Serous effusions. *See* edema.
Ship beriberi, 24, 127.
Skin lesions, 5, 6.
Smith curative unit, 169, **213–216.**
Sources of vitamin B_1, **226–242.**
Species classification, 314.
  requirement for vitamin B_1, **309–315.**
  size and time of onset of polyneuritis, 312–314.
  size and vitamin B_1 requirement, 309–315.
Specificity of function of thiamin, **331–334.**
Spontaneous cures, 208.
Sporadic cases of beriberi, 26.
Sprue, 3, 6, 13, **45,** 61, 85.
Standards of thiamin, 168, 169.
Staphylococcus aureus, 181, 191, 343, 383.
Starch, corn, dextrinized, 219.
  diet, producing beriberi, 27.
Stomach preparations, 6, 92.
Stomatitis, 5.
Storage of glycogen, **316–330.**
  vitamin B_1, **243–250.**
  vitamins, 4, 18.
Streptothrix, 382.
Structure of riboflavin, 132.
  thiamin, **144–150.**
Subclinical forms, 3, 7, 64, 84, 87.
Subcutaneous injection 39, 79–82.
Substituted pyrimidines, **180–190,** 384–385.
  thiazoles, **191–196,** 384–385.
Sugar, vitamin B_1 in, 234, 240.
Sulfite cleavage of thiamin, **146–149.**
  destruction of thiamin, 161.
Sulfur in thiamin molecule, 140, 144, 146.
Sunflower seed, 201.
Supplements, dosage of, 88.
  vitamin containing, 84, **85,** 88.
Sweat, 253.
Symbiosis, 374–375, 385.
Symptoms, correlation with degree of deficiency, 20.
  early. *See* prodromal symptoms.
Synonyms for beriberi, 16.
  vitamins, 4.
Synthesis of thiamin, 150–155.
  Andersag-Westphal, 153–155.
  Todd-Bergel, 154–155.
  Williams-Cline, 150–153.
  by bacteria, 378.
  by plants, 180, 383–384.
  in leaves, 380–381.

Tachycardia, 19, 20, 21, 23, 80.
Technique for minute amounts, 140–142.
Temperature, subnormal, 337.
Tenderness of muscles, 19, 21.
Tests for thiamin, 87, 165–168. *See also* analysis.
Tetany, 5.
Therapeutic utility of thiamin, 333–334. *See also* clinical studies.
Thermolabile factors, 133.
Thermostable factors, 131, 133, 302.
Thermostability, **130–131, 158–161.**
Thiamin and riboflavin, 328.
  as activator of carboxylase, 345.
  as nutrilite, 371–390.
  bromide hydrobromide, 153.
  -calorie ratio, **95–104,** 107, 108, 110, 258–260.
  -calorie ratio for species, 313.
  -carbohydrate ratio, **100–104.**
  catatorulin action of, 337–340.
  chloride hydrochloride, also called thiamin chloride, or thiamin hydrochloride, 166.
  deficiency, degree of, 20.
  free base of, 163–164, 167.
  general role of, 371–390.
  intermediates, bioactivity of, 383–384.
  isolation, **138–143.**
  -non-fat-calorie ratio, **101–104,** 107.
  origin of name, 134, 135.
  pyrophosphate, 172–173, 337, 343, **344–346.**
  pyrophosphate, occurrence, 173, 345.
  pyrophosphate, synthesis, 345.
  quaternary nature of, 148, 149.
  synonym for vitamin B_1. *See* special topics, *e.g.*, assay, clinical studies with, formula, occurrence, etc.
Thiazole cleavage products of thiamin, 147, 148.
  derivatives, 191–196.
  intermediate of thiamin, 147, 148, 150, 191.
  nucleus, 144, 148.
  nucleus, occurrence in nature, 144, 148.
  related to thiamin, **191–195.**
  synthesis of, 150, 193, 194.
Thiochrome, 161, **174, 175.**
  method of assay, 87, 175, 198, **217–218.**
  reaction, 175.
Thujone convulsions, 81.
Thyroxin, 79, **262,** 360–361.
Tikitiki extract, 7, 88, 89.
Tissue oxidases, 335–337.
  respiration, 331–355.
  respiration, reconciliation of theories, 349–352.
Tissues, carbohydrate metabolism in, 332–334.
  distribution of vitamin B_1 in, **243–250.**
  oxidation in, 337.
Titration of thiamin, potentiometric, 148, 149, 163.
Tolerance of thiamin in man, 81.
Tomato roots, 380, 383.
Torula, 180, 383–384.
Toruline, 126. *See* thiamin and vitamin B_1.
Toxemia in pregnancy, 41, 43.
Toxicity, cumulative, 82, 286–287.
  of beriberic milk, 363.
Toxicology, **81–82,** 286–287.
Treatment, clinical, response to, 90.
  general principles of, 87.
  method of, 88.
  of coexistent diseases, 90.
  of thiamin deficiency, 86–90.
  specific, **88, 89.**
  symptomatic, 89.
Tuberculosis, neuritis of, 49, 61, 85.
Tumors, 50, 61, 85.
Turkey dermatitis, 132, 133.
Typhoid fever, 11.

Ulceration of intestine, 5, 31, 47.
  mouth, 5, 6.
  skin. *See* skin lesions.
  stomach, 69.
Ultraviolet absorption, 146, 149–152.
  of thiamin, 154.
Unavailability of vitamin in International Standard Clay, **213–216.**
Units, vitamin B_1, 91, 168–169, **213–216.** *See also* International, Chase-Sherman, Roscoe, Smith curative and milligram equivalent.
  conversion of, 91, 169.
Urinary excretion, diminished in beriberi, 19, 23, 102. *See also* excretion of vitamin B_1.
Urine, assay of vitamin B_1 in, 87, **217–218.**
  excretion of vitamin B_1 in, 87, 102, **250–253.**
Utilization of thiamin, 18.

Vagi, 21.
Vagotonia, 80.
Vagus nerve, 65.
Variation in requirement, 90. *See also* requirement.
Vaso-dilatation, **64–67,** 80.
Vegetables, vitamin B_1 in, **233.**
Vegex, 38, 39.
Venesection, 89.
Virus diseases as cause of neuritis, 9.
Vitamin(e), 126.
Vitamin A, 6, 13, 14, 127, 128, 359.
Vitamin B, 3, 134. *See also* vitamin B complex.
Vitamin B complex, 3, 4, 13, **130–135.** *See* frontispiece.
Vitamin B_1. *See* special topics, *e.g.*, assay, clinical studies with, formula, occurrence, etc. *Also* Thiamin.
isolation of, **138–143.**
preparations of, 90–92.
requirement. *See* requirement.
sparing action of fats, 101.
B_2, 4, 131. *See* riboflavin.
B_2 complex, 4, 131, 133–134. *See also* vitamin B complex.
B_3, 4, 133, 300 301.
B_4, 4, 133, 204, 284.
B_5, 4, 134, 204.
B_6, 4, 131, 132, 134.
C, 4, 25, 79, 336, 371.
D, 4, 128.
G, 131. *See* riboflavin.
-calorie ratio, 37, **95–104,** 258–260. *See also* thiamin-calorie ratio.
Vitamins and microorganisms, 371–375, 378–379, 382.
and plants, 371–382.
balance among, 3.
category of, 3, 127–128.
discovery of, **123–129.**
dissimilarity of, 128.
Vomiting, 4, 22.

W factor, 133.
Weakness. *See* Asthenia.
Weight and rapidity of onset of beriberi, **304–307.**
and vitamin B_1 requirement. *See* requirement.
maintenance and vitamin B_1 intake, **300–304.**
maintenance method of assay, 197, **206.**
restoration methods of assay, 198, **206, 207.**
Wet beriberi, 16, 20, 68.
Wheat, vitamin B_1 in, **231–233,** 238.
Wheat germ, 7, 92, 109.
use as therapeutic substance, 88.
Williams-Waterman factor. *See* vitamin B_3.

Yakriton, 363.
Yeast, 7, 92, 109, **235–236.**
growth of, 373–375.
method of assay, 198, **220.**
preparations, 6.
use as therapeutic substance, 88.

Zeolite as an adsorbent, 140, 217–218.

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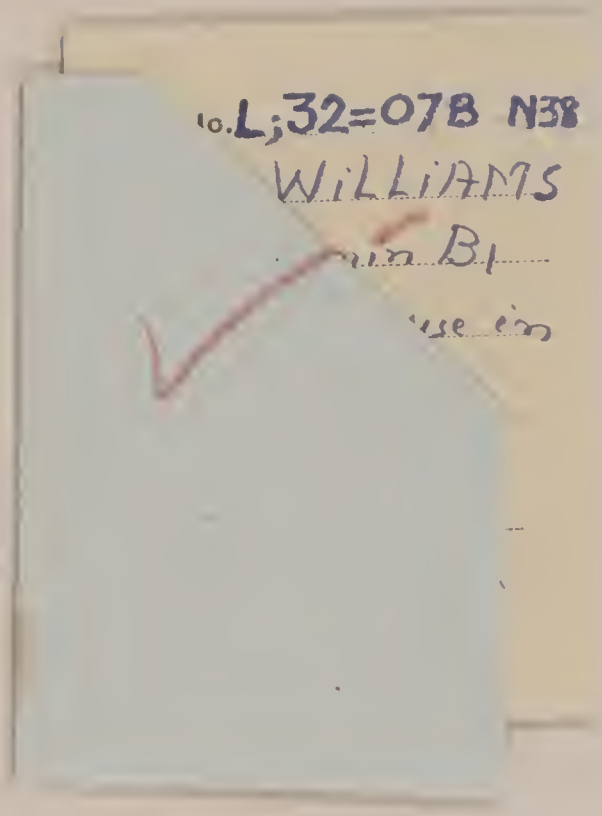
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